

# **New Indigo workshop on “Antiparasitic and Antitumour drugs”**

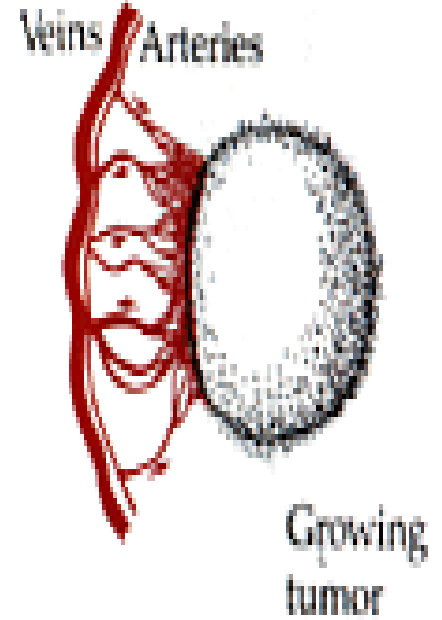
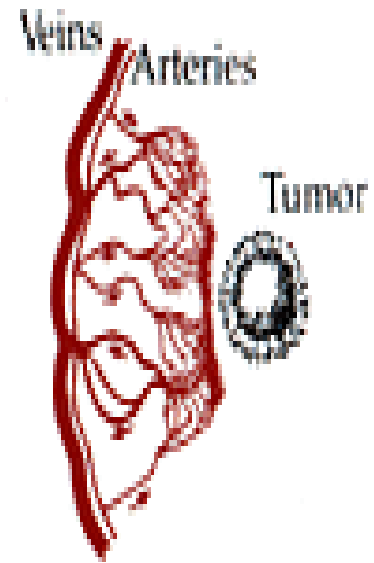
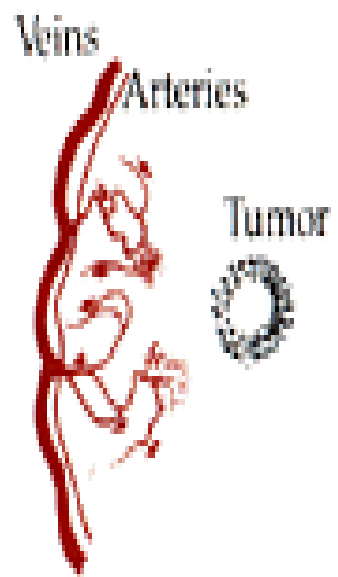
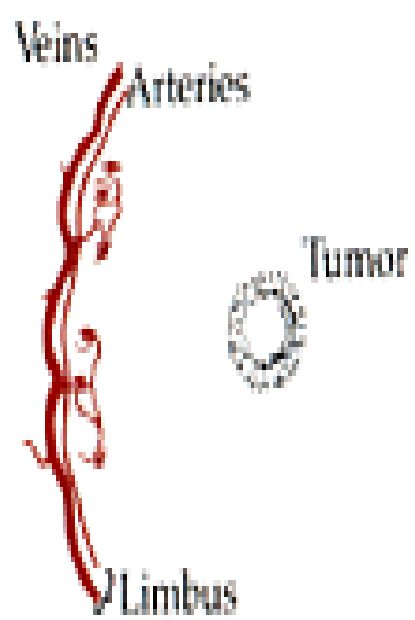
IBMC, 9<sup>th</sup> September, 2011

## Targeting angiogenesis in cancer

Raquel Soares

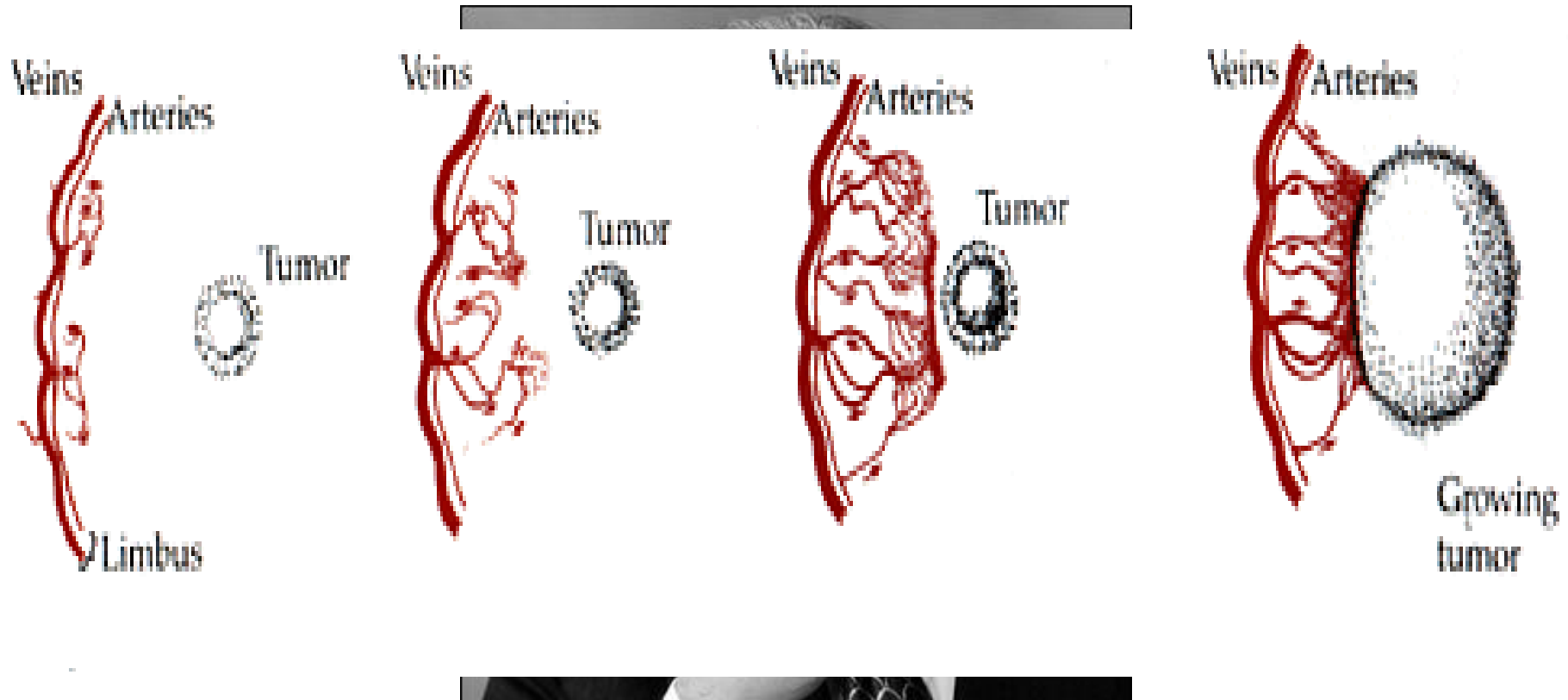
Department of Biochemistry





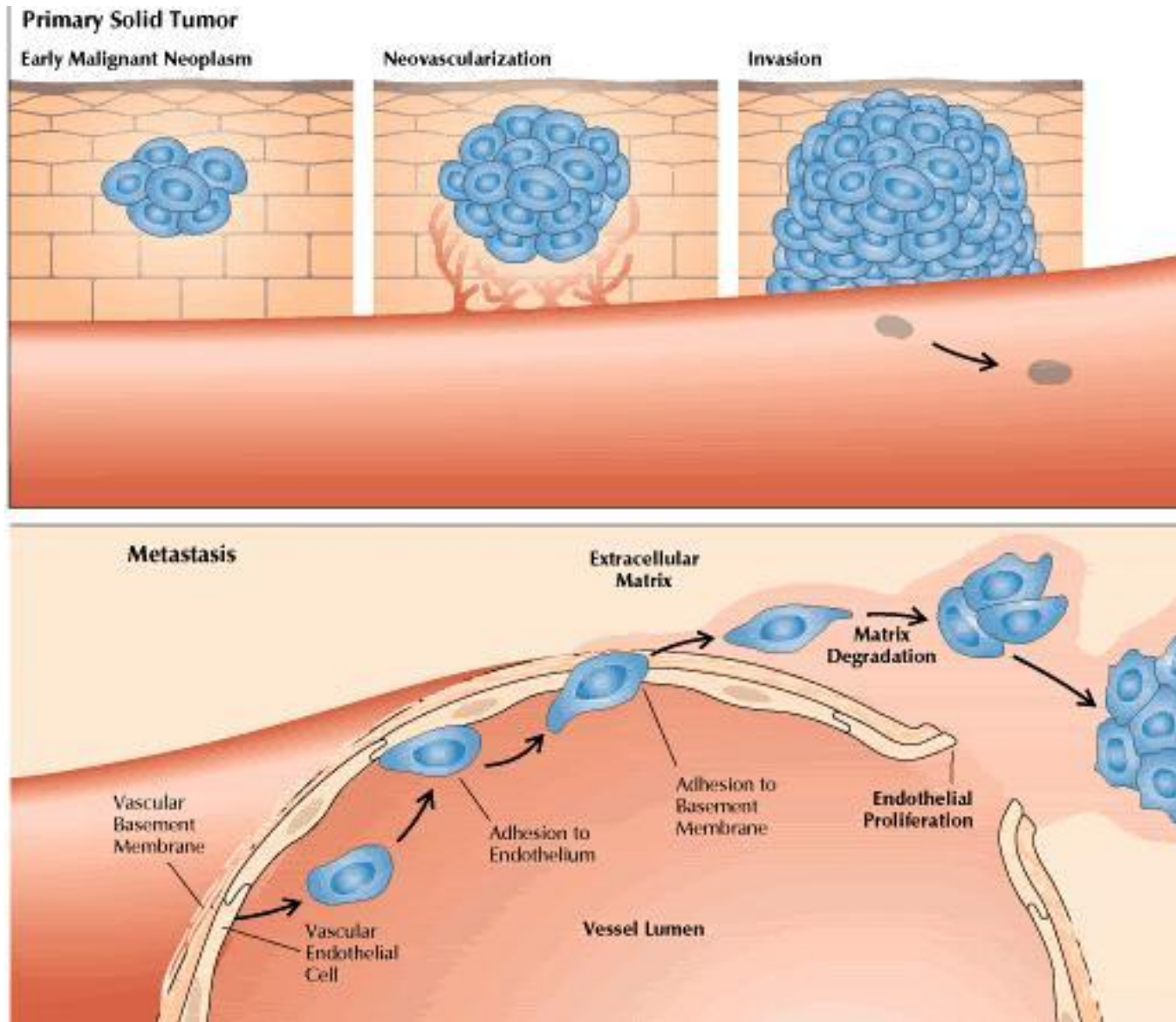
# Tumor angiogenesis: therapeutic implications

*J Folkman. N Engl J Med, 1971; 285: 1182-6.*



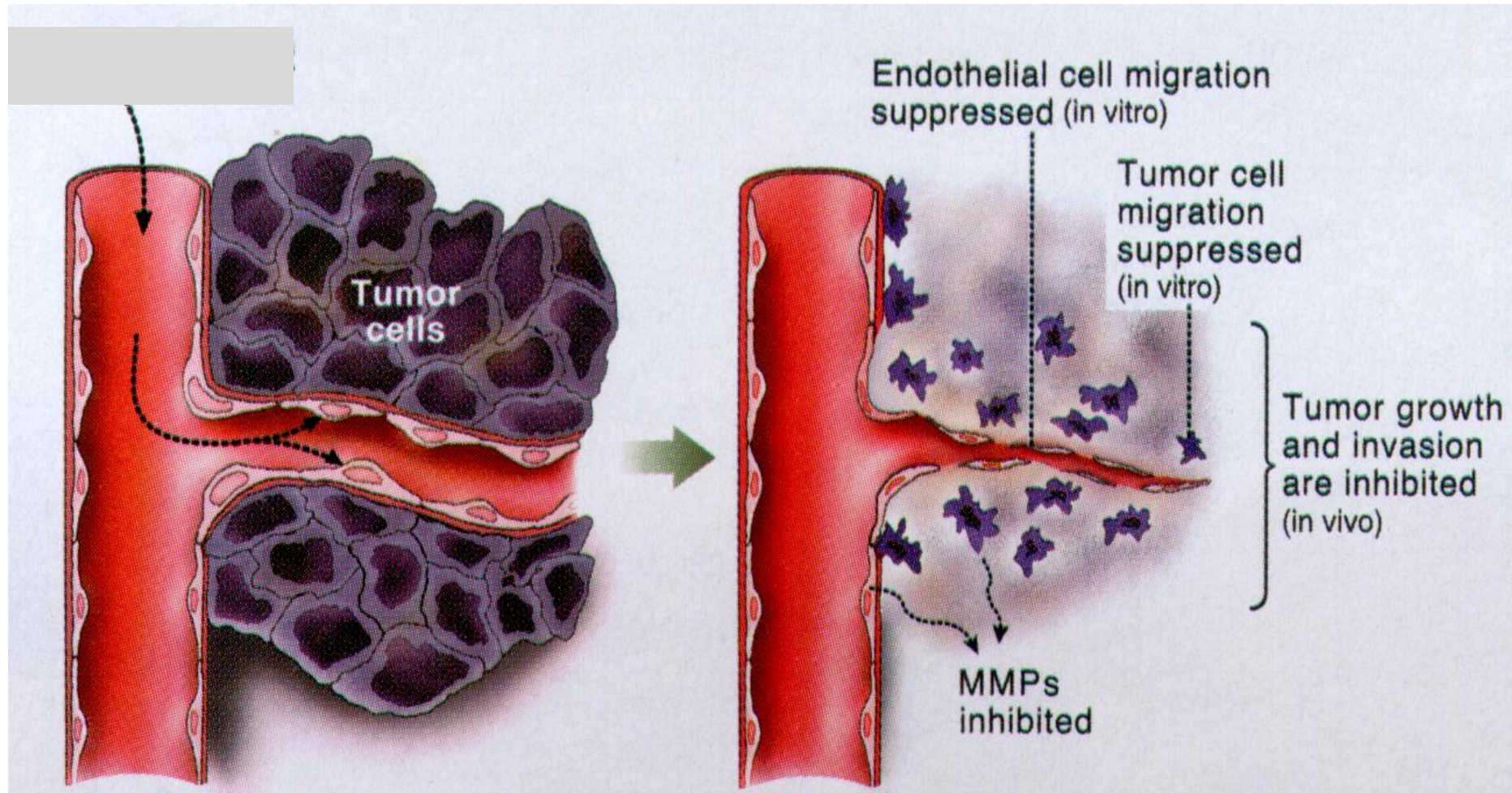
Angiogenesis is required for tumors to grow beyond 1-2 mm<sup>3</sup>

# Angiogenesis results in Metastization



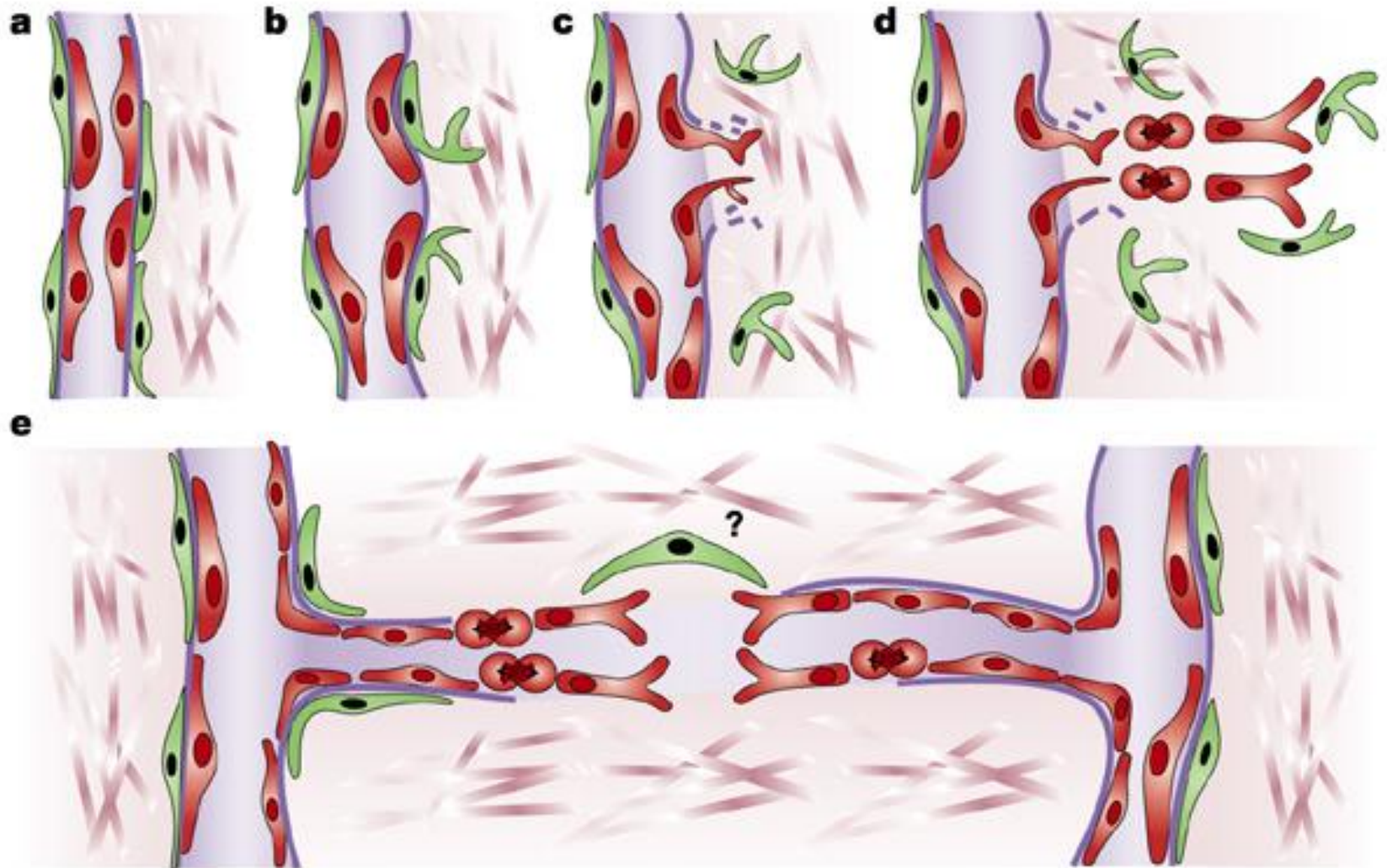
# Tumor angiogenesis: therapeutic implications

*J Folkman. N Engl J Med, 1971; 285: 1182-6*



Angiogenesis blockage can be a good strategy to prevent tumor growth

# Angiogenesis is a complex process



**Inhibitors:**

Thrombospondin-1

*The statins:*

Angiostatin

Endostatin

Canstatin

Tumstatin

**Activators**

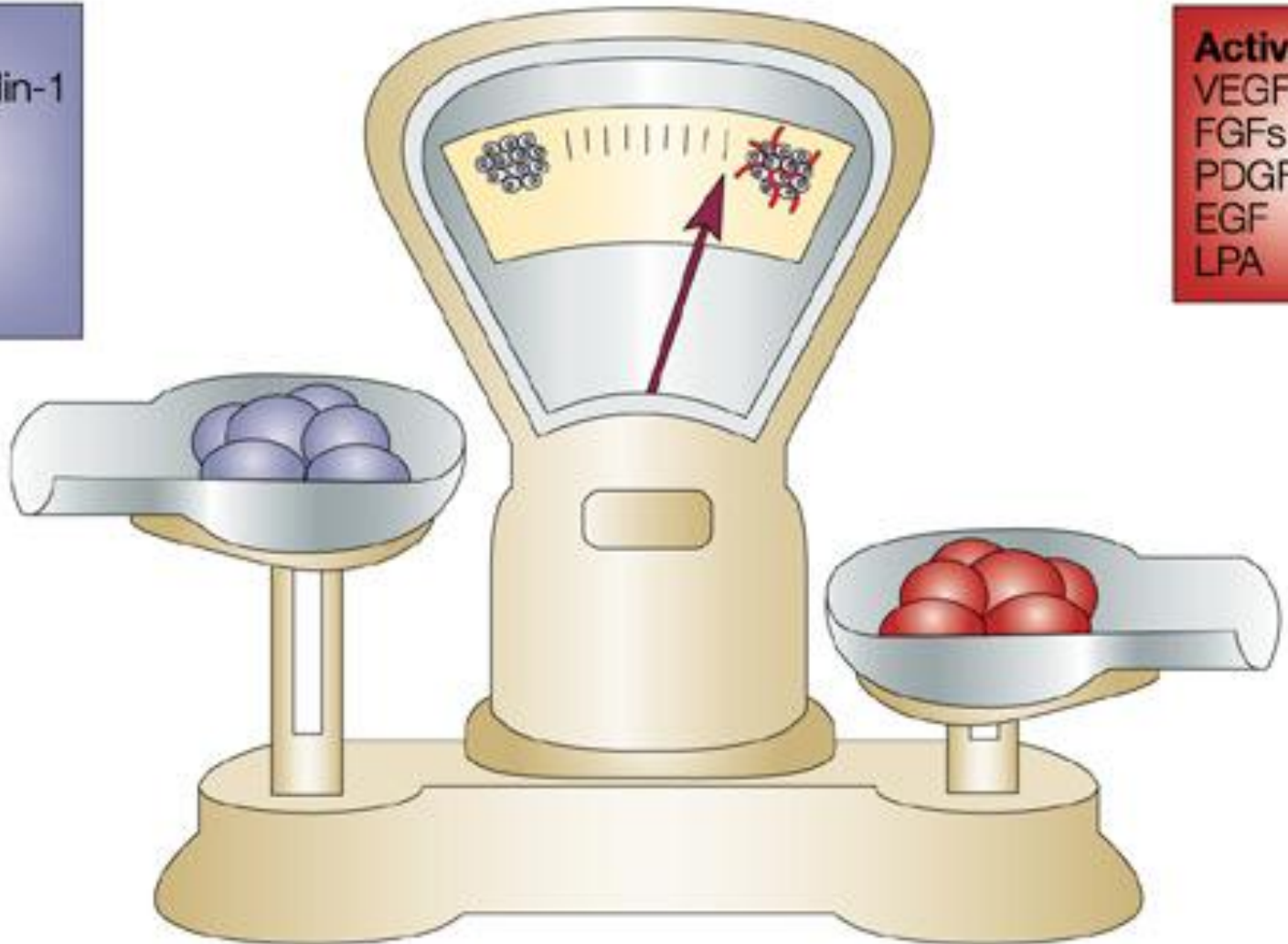
VEGFs

FGFs

PDGFB

EGF

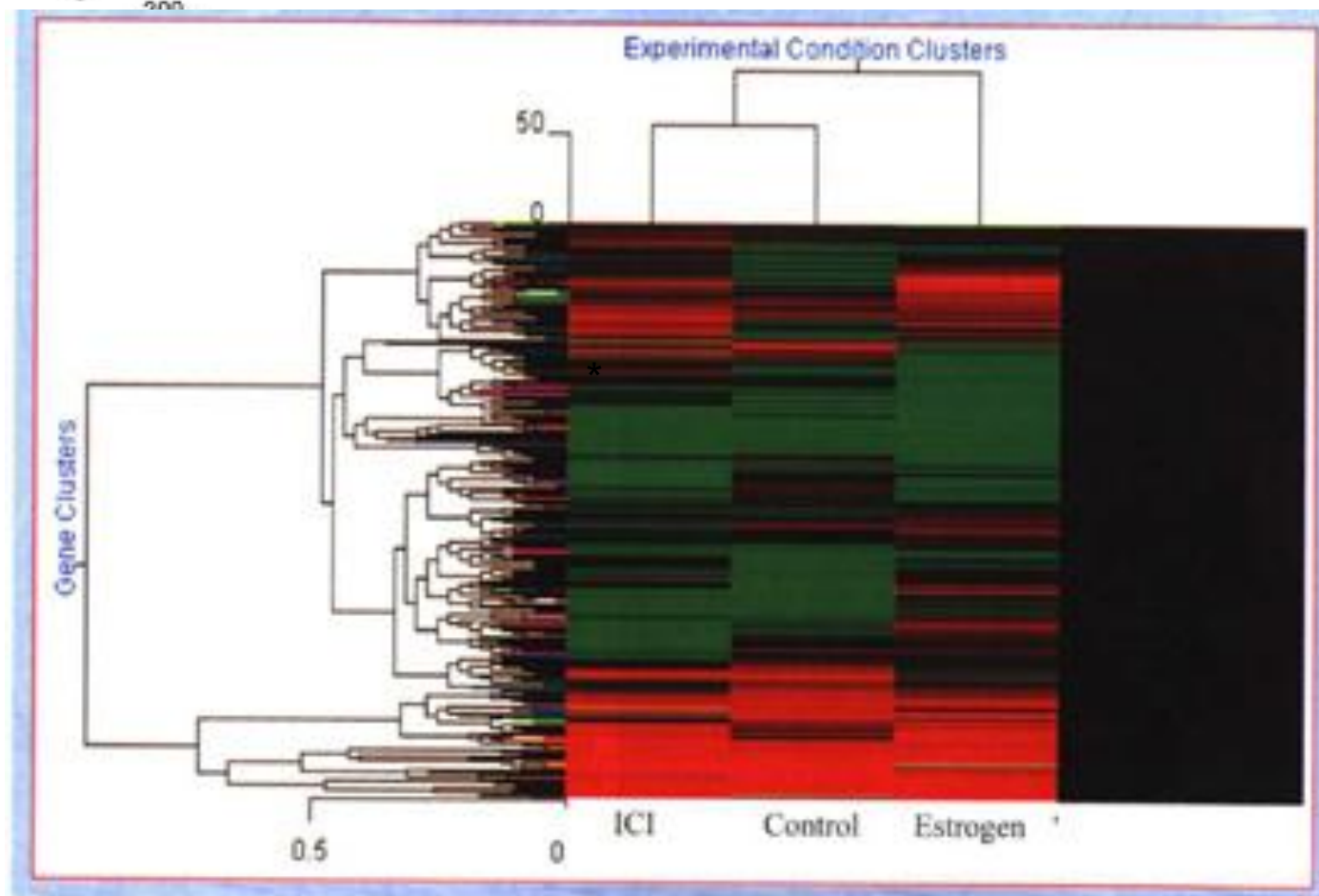
LPA



1) Estrogens as potential targets for angiogenesis inhibition

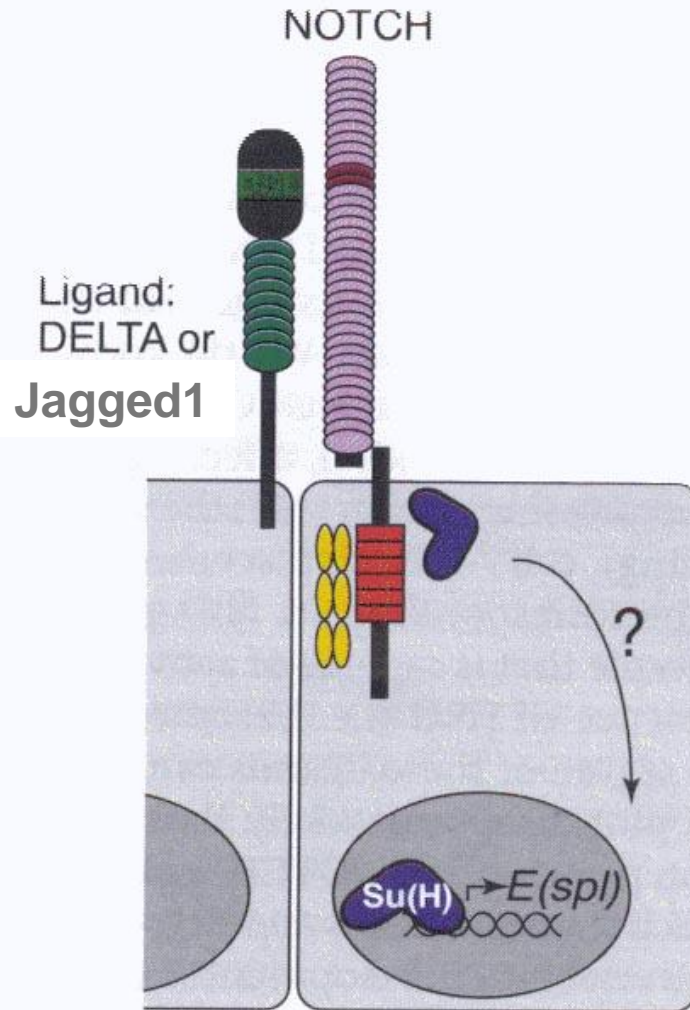


# Estradiol induces Notch1 expression and activity, **enhancing angiogenesis** in breast cancer *in vitro* and *in vivo*



17β-e

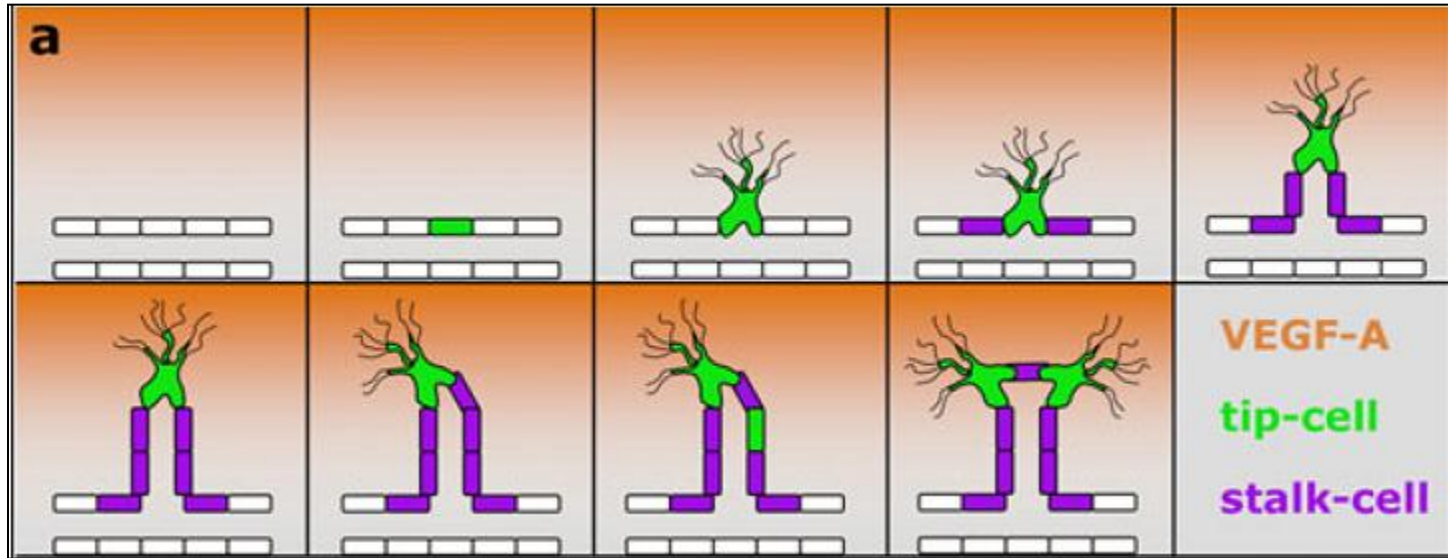
# Notch signaling pathway



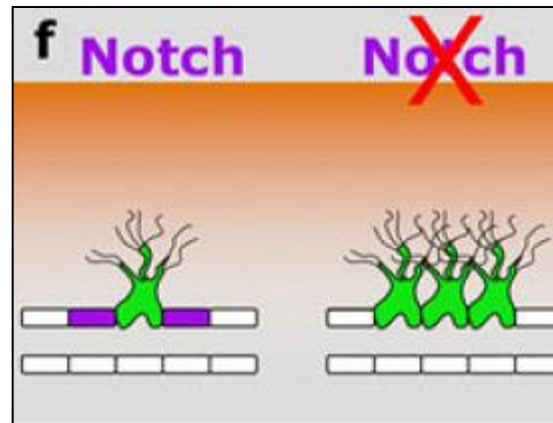
## Notch:

- Highly conserved transmembrane receptor family (Notch1-4)
- Regulates cell migration in embryogenesis
- Is involved in angiogenesis

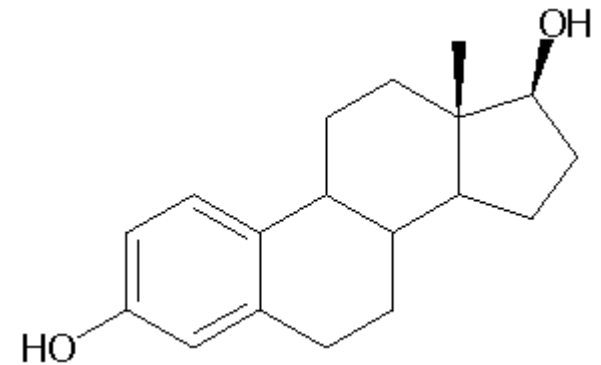
# NOTCH signaling inhibits tip cell response in stalk cells



Tip cell numbers further increase through slightly elevated growth



Conclusions:

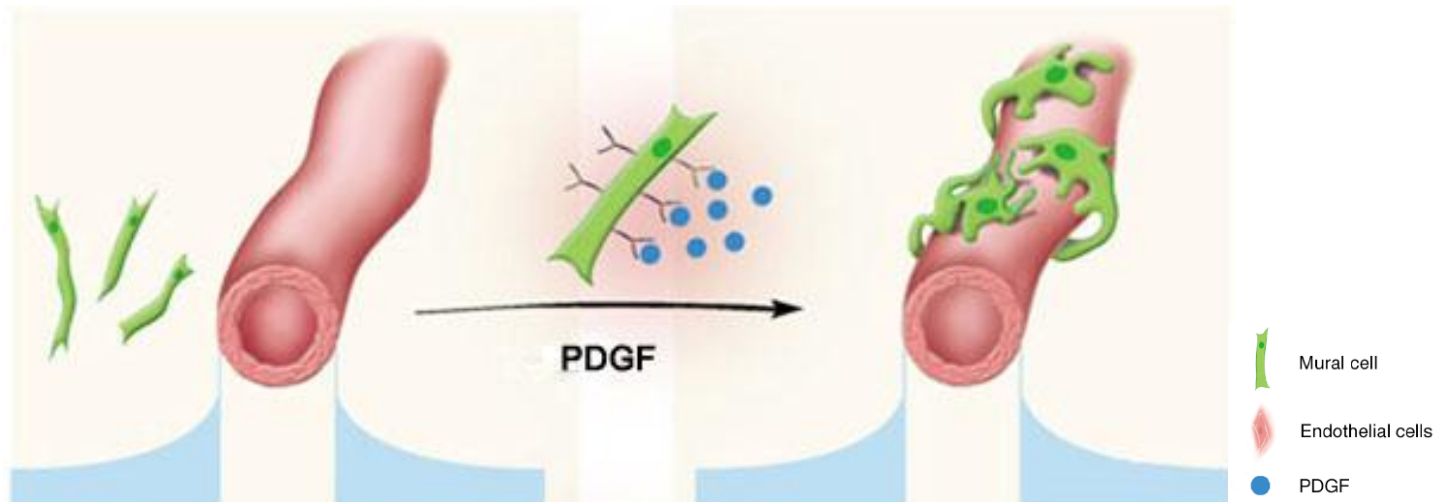


Estradiol

induces expression of Notch1 in EC

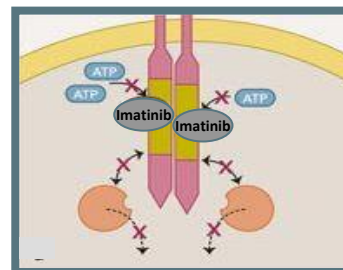
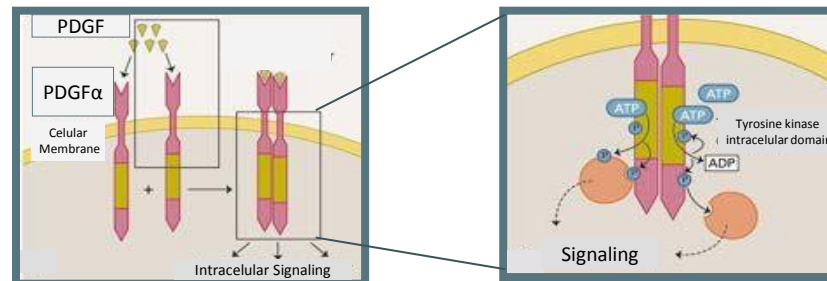
enables the assembly of normalized  
functioning vessels

# Mural cells' recruitment is a PDGF gradient-dependent event

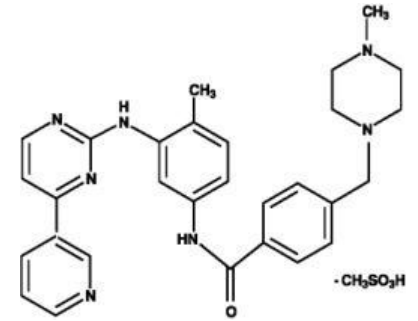
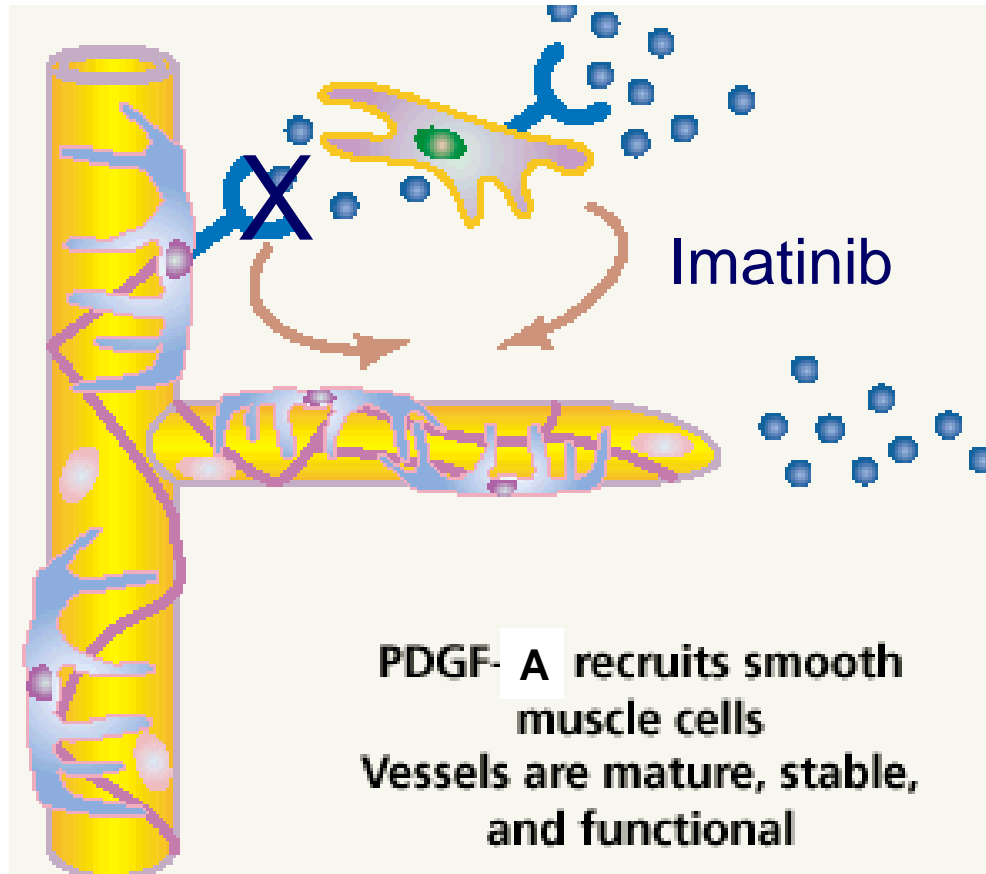


# IMATINIB

- Also known as Gleevec<sup>®</sup>, Glivec<sup>®</sup> or STI571
- Potent inhibitor of tyrosine kinase receptors such as **PDGFR- $\alpha$**  and **- $\beta$**   
c-Kit  
Bcr-Abl protein
- FDA approved for chronic myeloid leukaemia (CML)  
gastrointestinal stromal tumours (GIST)



# Imatinib inhibits PDGF signaling in Smooth Muscle Cells



2) Is Imatinib an angiogenic mediator?

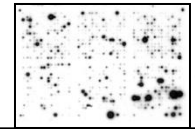


## Elucidating Progesterone Effects in Breast Cancer: Cross Talk With PDGF Signaling Pathway in Smooth Muscle Cell

Raquel Soares,<sup>1\*</sup> Susana Guerreiro,<sup>1</sup> and Mónica Botelho<sup>2</sup>

<sup>1</sup>Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

<sup>2</sup>IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal



cDNA array

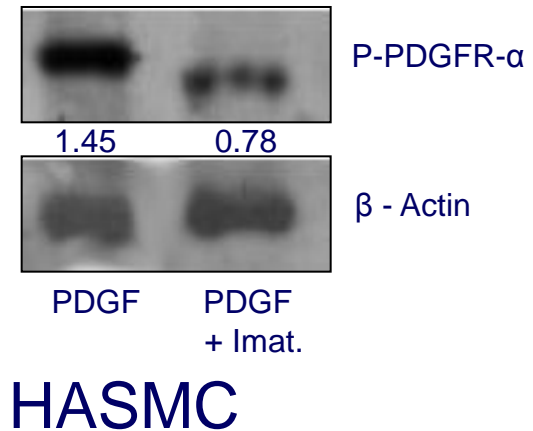
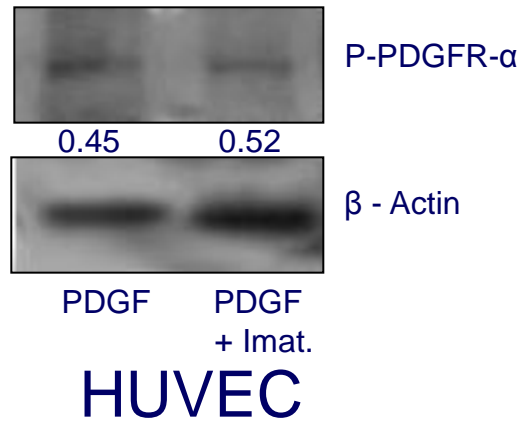
Gene name	Progesterone/control ratio
DNA repair	7.13
BARD	6.21
Histone H4	5.91
Aurora-associated protein	5.45
<b>PDGF-associated protein</b>	<b>5.44</b>
Platelet-basic protein	4.77
<b>PDGF-A</b>	<b>4.61</b>
AP4 basic	4.23

Angiogenesis (2007) 10:279–286  
DOI 10.1007/s10456-007-9080-2

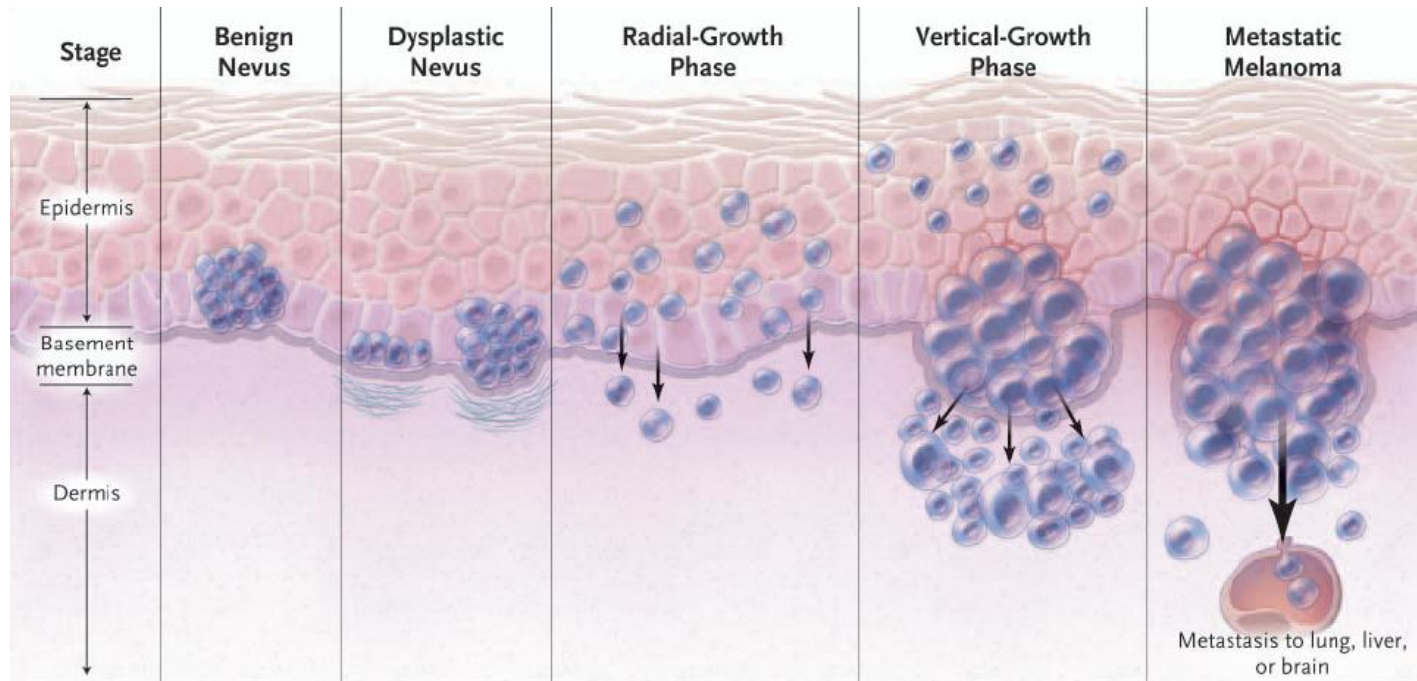
ORIGINAL PAPER

## Anti-angiogenic effects of imatinib target smooth muscle cells but not endothelial cells

Ana Rocha · Isabel Azevedo · Raquel Soares



# MELANOMA: THE MODEL



**C-Kit**

**PDGF**

Miller and Mihm, 2006

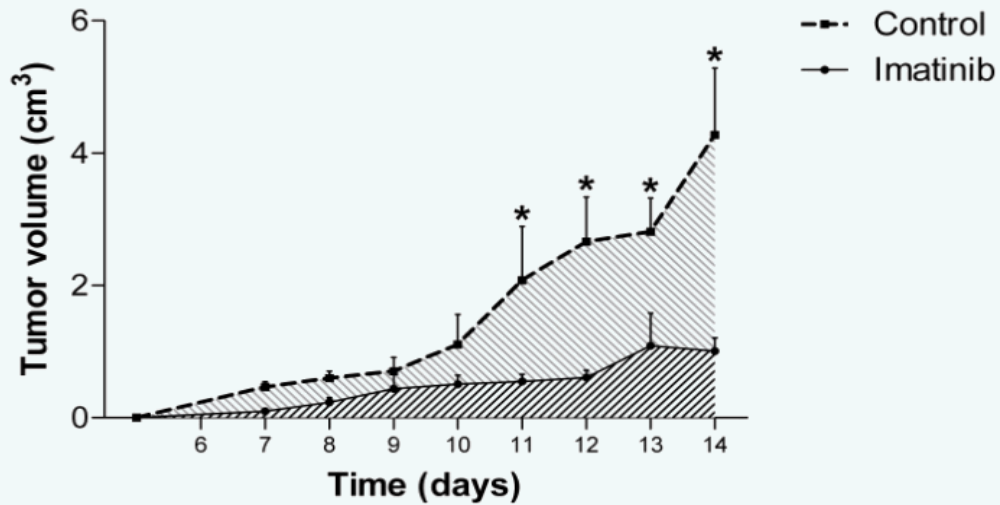
# AIM

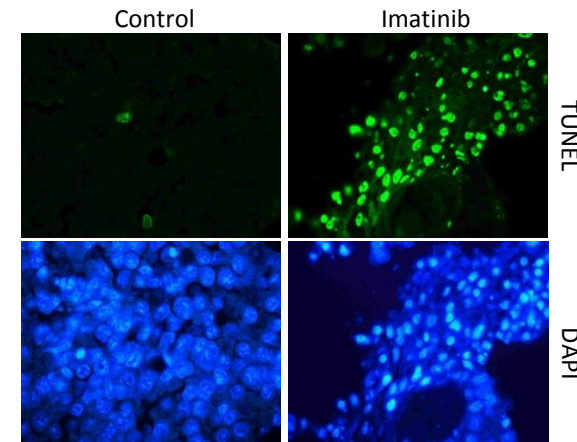
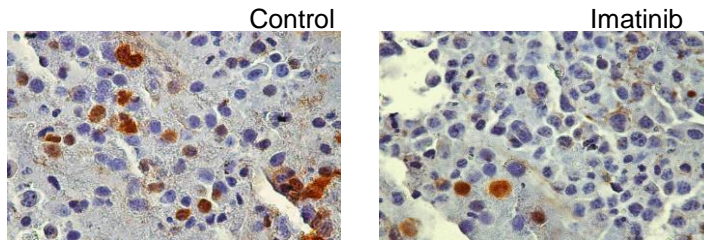
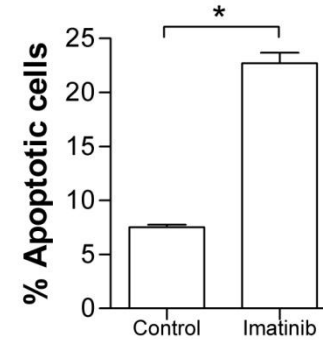
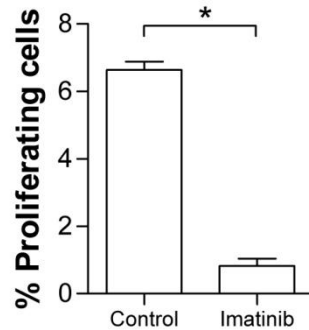
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Investigate the anti-angiogenic effect  
of imatinib on vascular endothelium and mural cells,  
taking melanoma as a model

C57Bl/6 mice inoculated with B16 mouse melanoma cell line

## TUMOR GROWTH

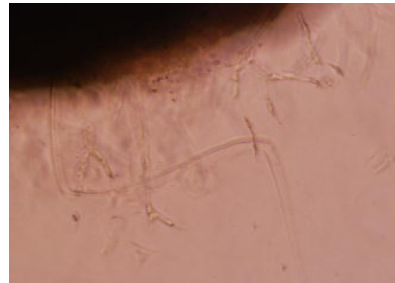




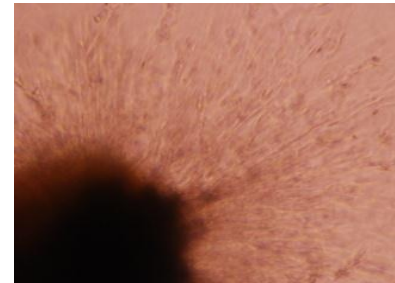
Imatinib therapy decreased proliferation and increased apoptosis on tumour cells

## Imatinib affects vasculature

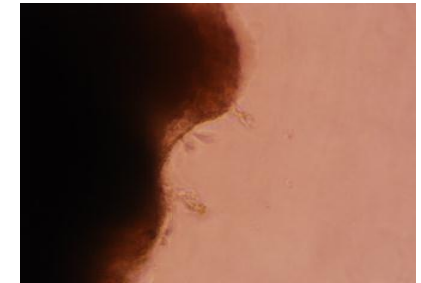
*Aortic ring assay*



Control

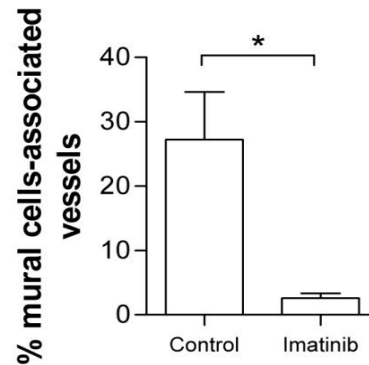
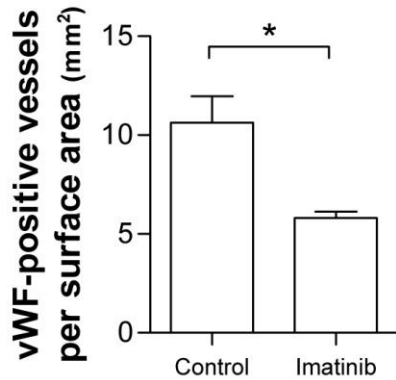


PDGF

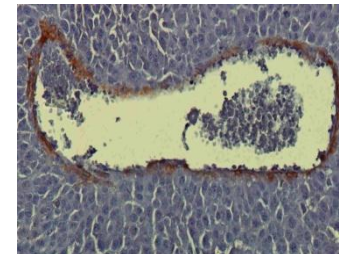


PDGF + Imatinib

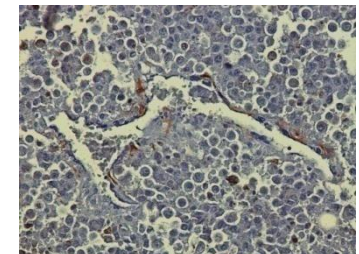
## Imatinib results in absence of support cells in mouse melanoma



aSMA staining

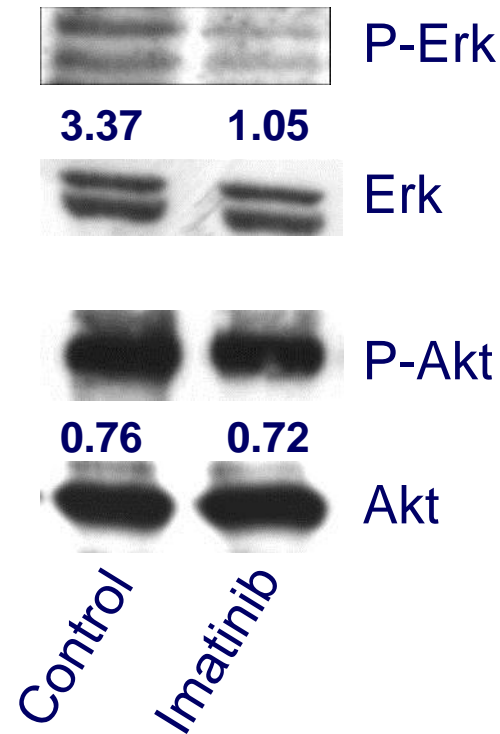
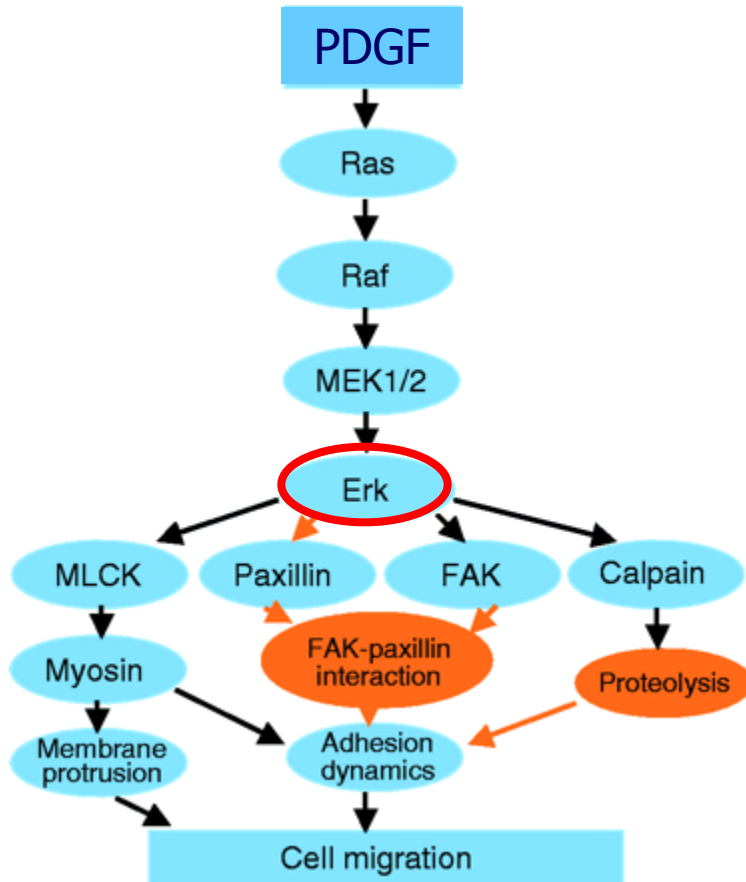


Control



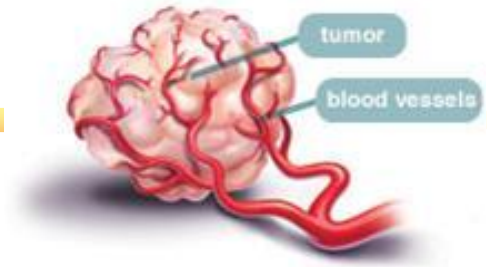
Imatinib

# Imatinib inactivates PDGFRa signaling (decreased P-ERK expression) in mouse melanoma



# CONCLUSION

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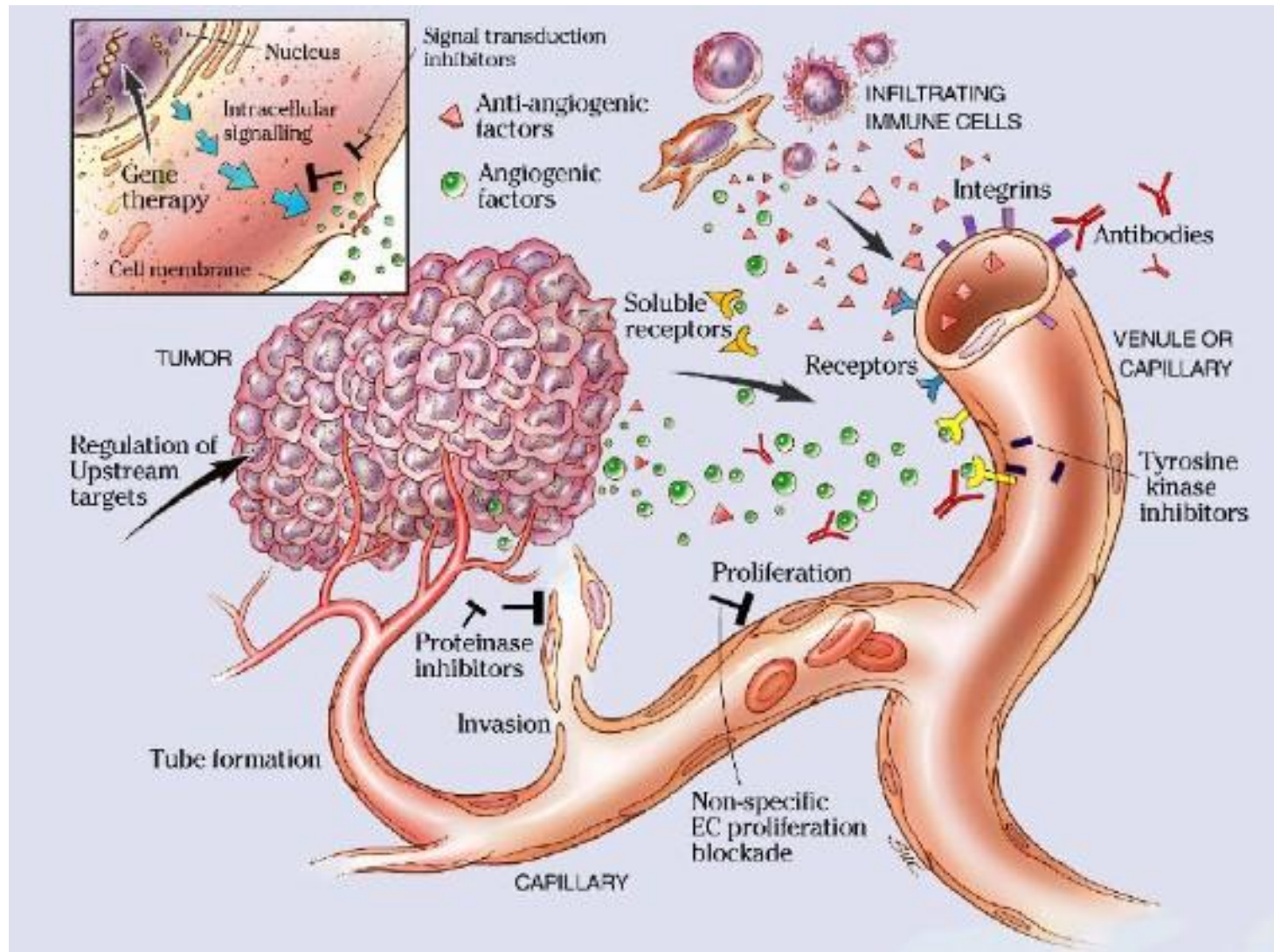
This work showed that imatinib:

- Abrogated B16 melanoma cells proliferation
- Increased B16 melanoma cells apoptosis
- Reduced vessels' number and decreased the percentage of mural cells-stabilized vessels

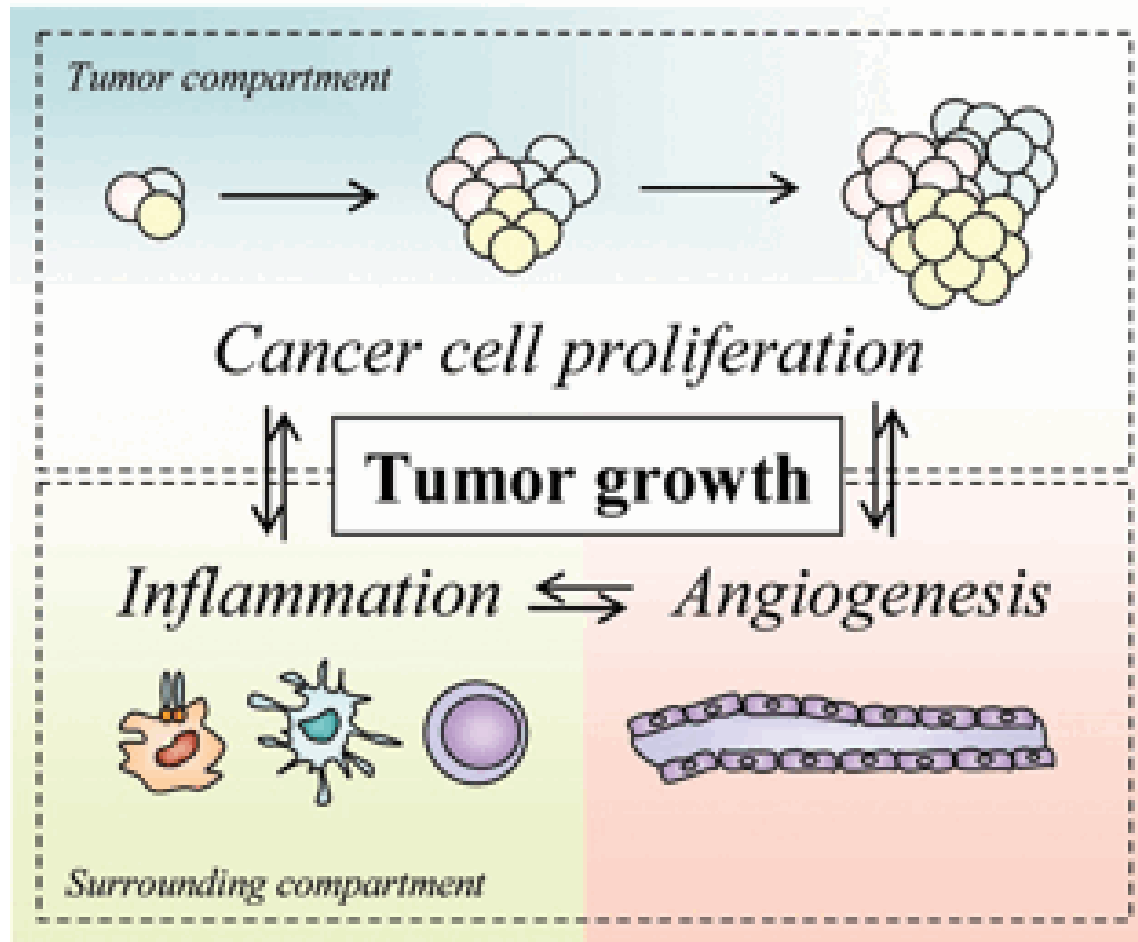
Imatinib has a double effect *in vivo*

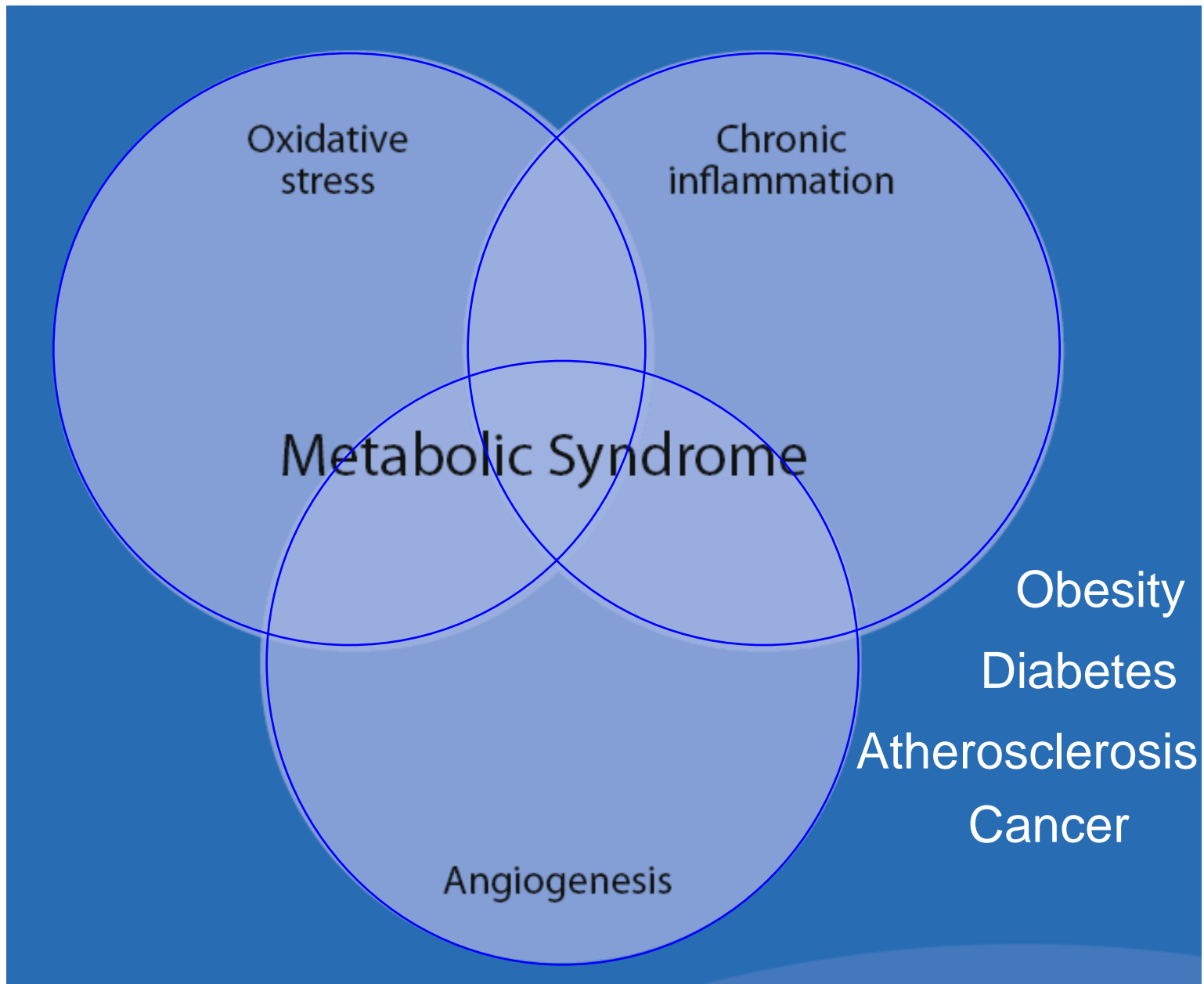


# Distinct cell types contribute to angiogenesis



# Angiogenesis and inflammation work together





Oxidative stress, inflammation and angiogenesis in metabolic syndrome.  
Edts Soares R and Costa C. Springer-Verlag, The Netherlands, 2009

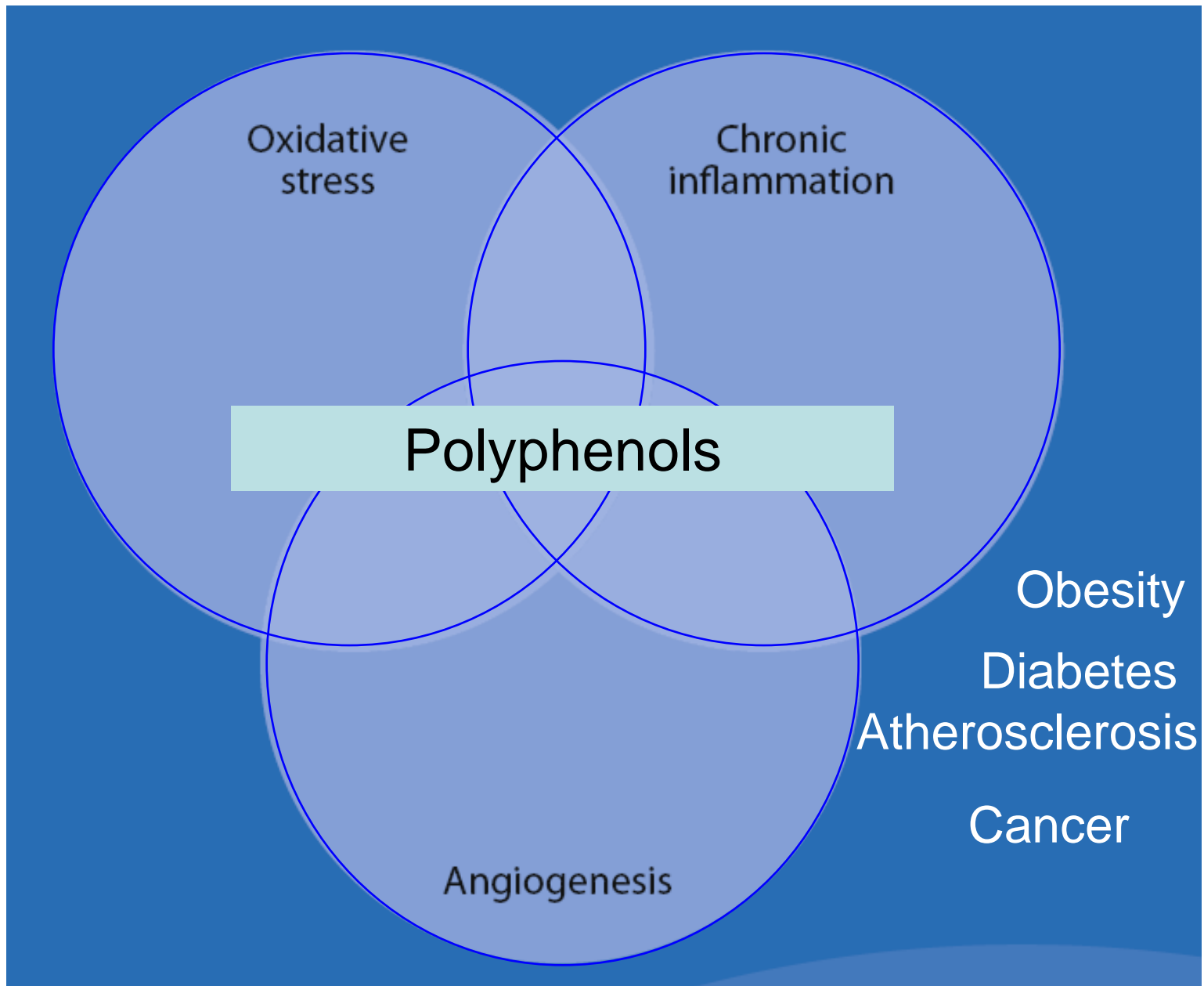
# Polyphenols



Present in diet (fruits, vegetables, beverages)

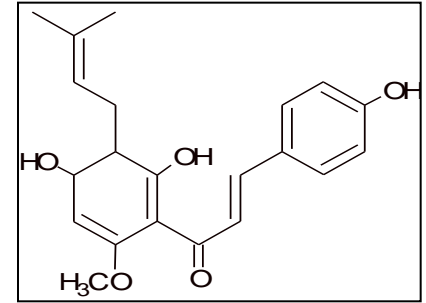
Anti-oxidant, Anti-inflammatory properties

3) Diet-derived polyphenols as potential anti-angiogenic agents



Oxidative stress, inflammation and angiogenesis in metabolic syndrome.  
Edts Soares R and Costa C. Springer-Verlag, The Netherlands, 2009.

# Polyphenols



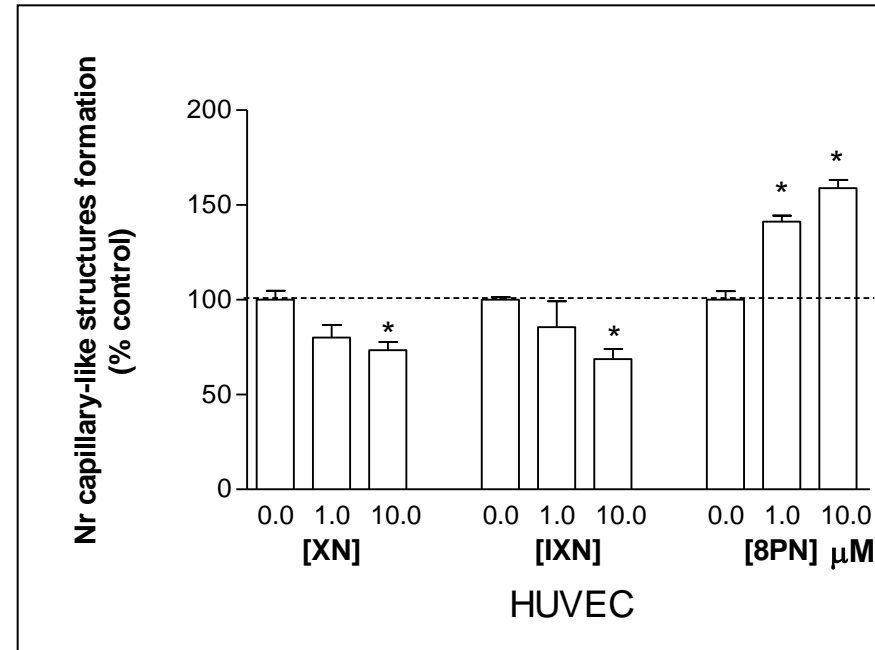
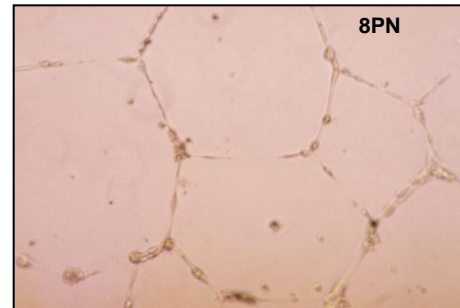
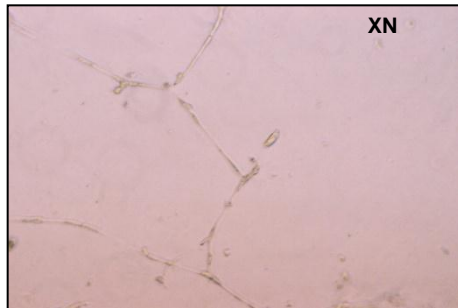
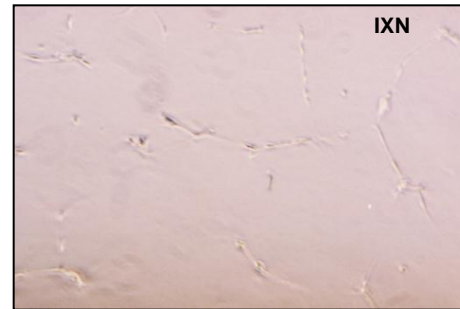
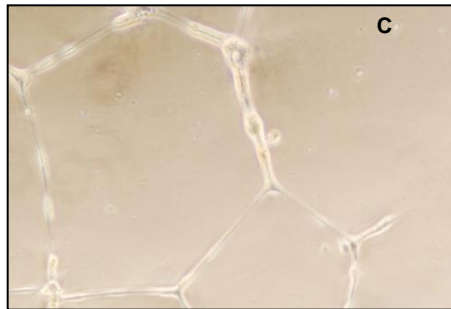
## Xanthohumol (XN)

- Prenylated chalcone
- Used in beer production (*Humulus lupulus*, L.)
- Metabolized to isoxanthohumol (IXN) e 8-prenylnaringenin (8PN)



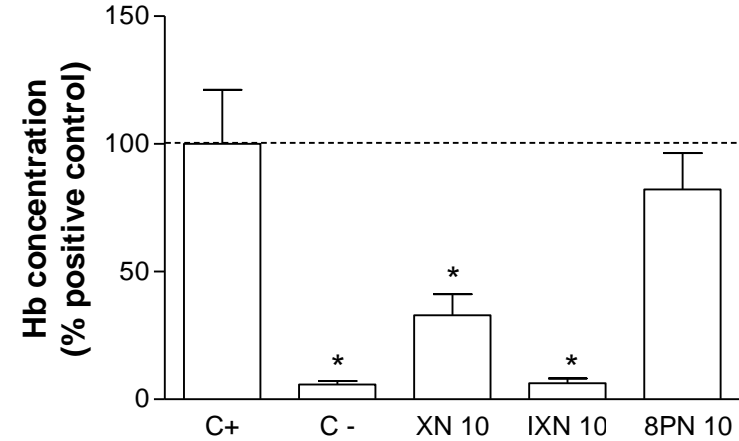
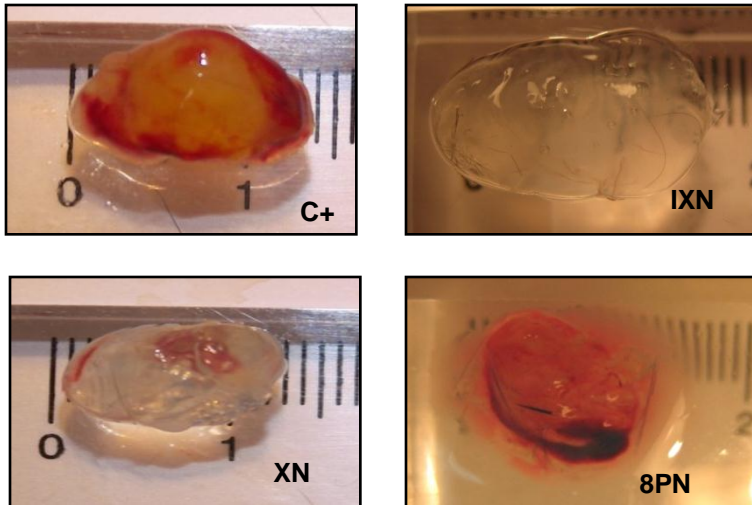
**Effects in angiogenesis?**

# XN, IXN and 8PN affect capillary-like structures formation

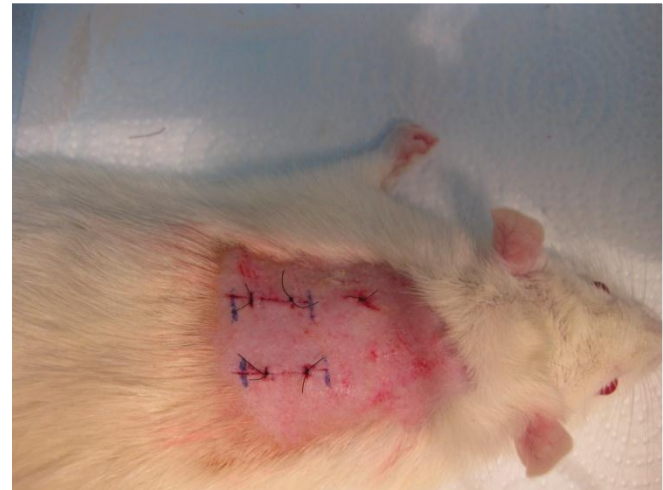




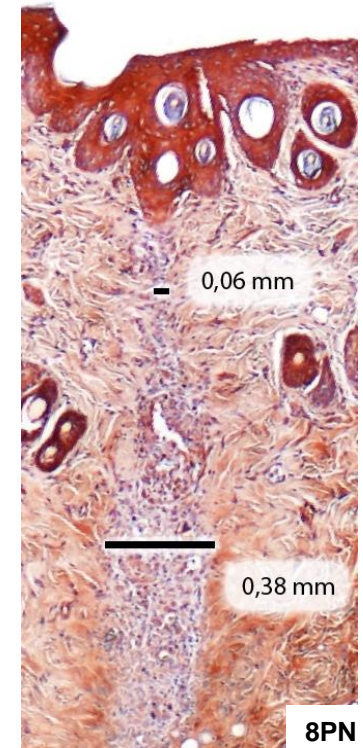
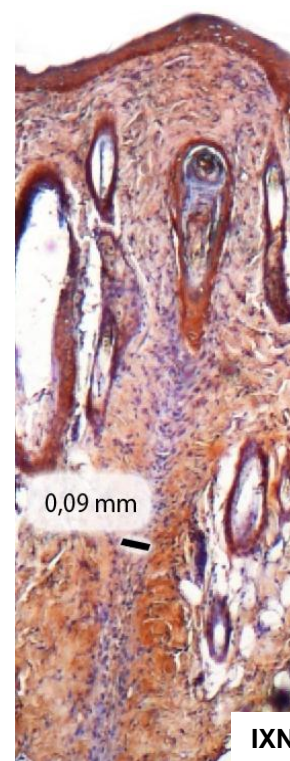
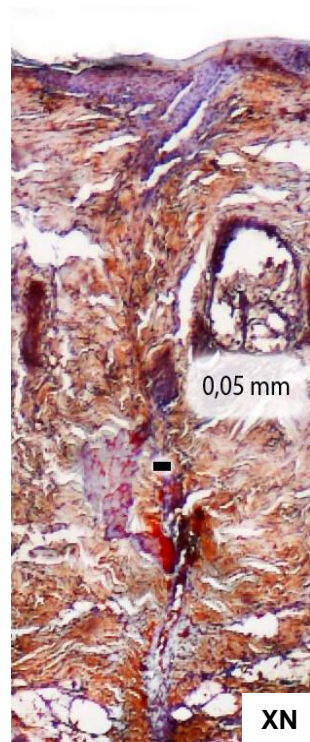
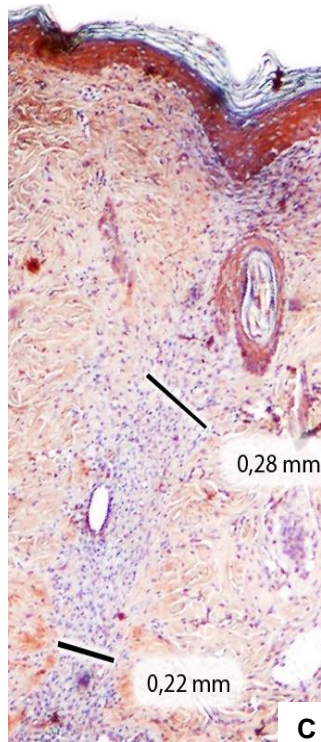
# Beer-derived polyphenols effects in angiogenesis in C57Bl/6 mice



# Effect of polyphenols in rat skin wound healing

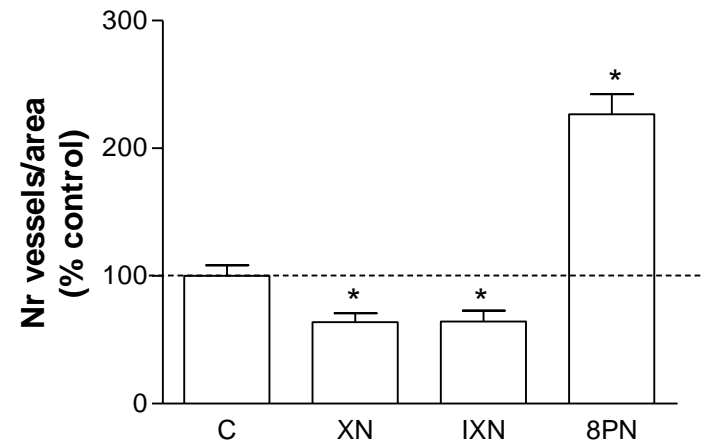
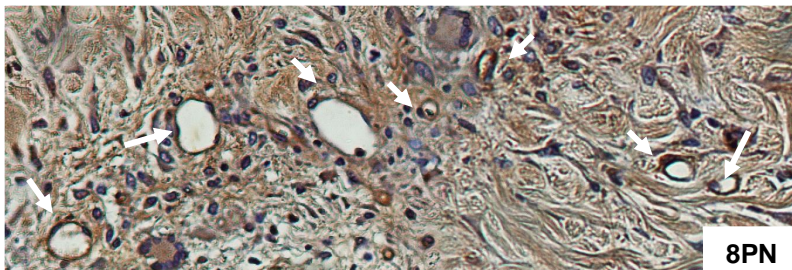
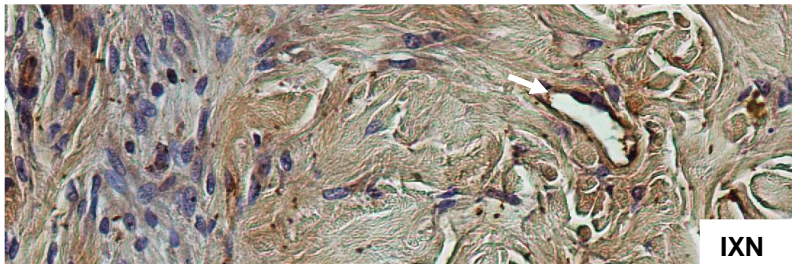
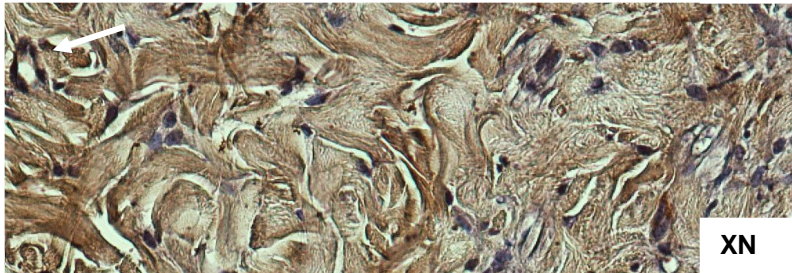
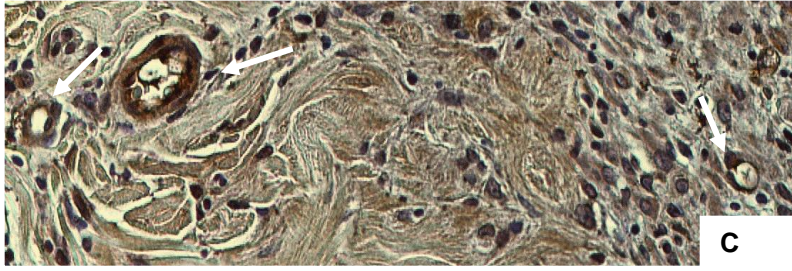


# Effect of polyphenols in rat skin wound healing



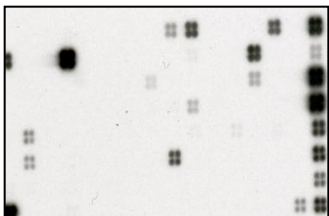
# Effect of polyphenols in rat skin wound healing

## Microvessel density

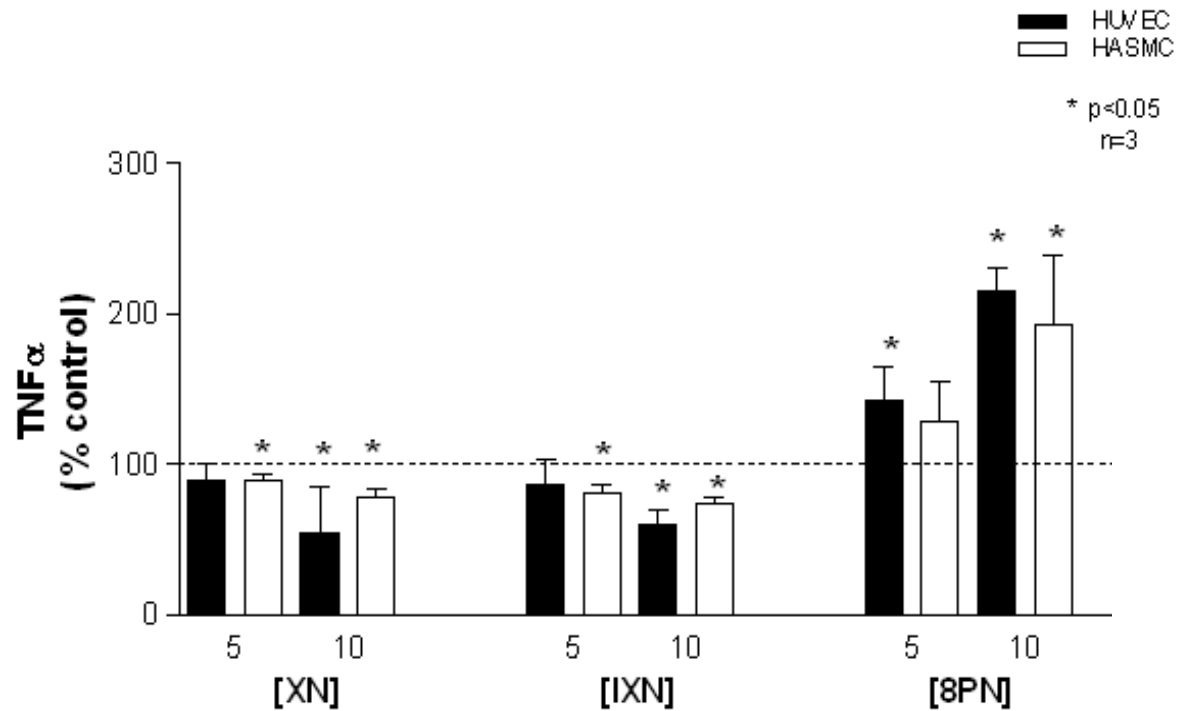


# XN, IXN and 8PN interfere with gene expression profile in endothelial and smooth muscle cells

Gene	HUVEC			HASMC		
	XN	IXN	8PN	XN	IXN	8PN
IL6			↑			↑
TNFRS12A	↓	↓				
IL12	↓	↓		↓	↓	
Ang2			↑			

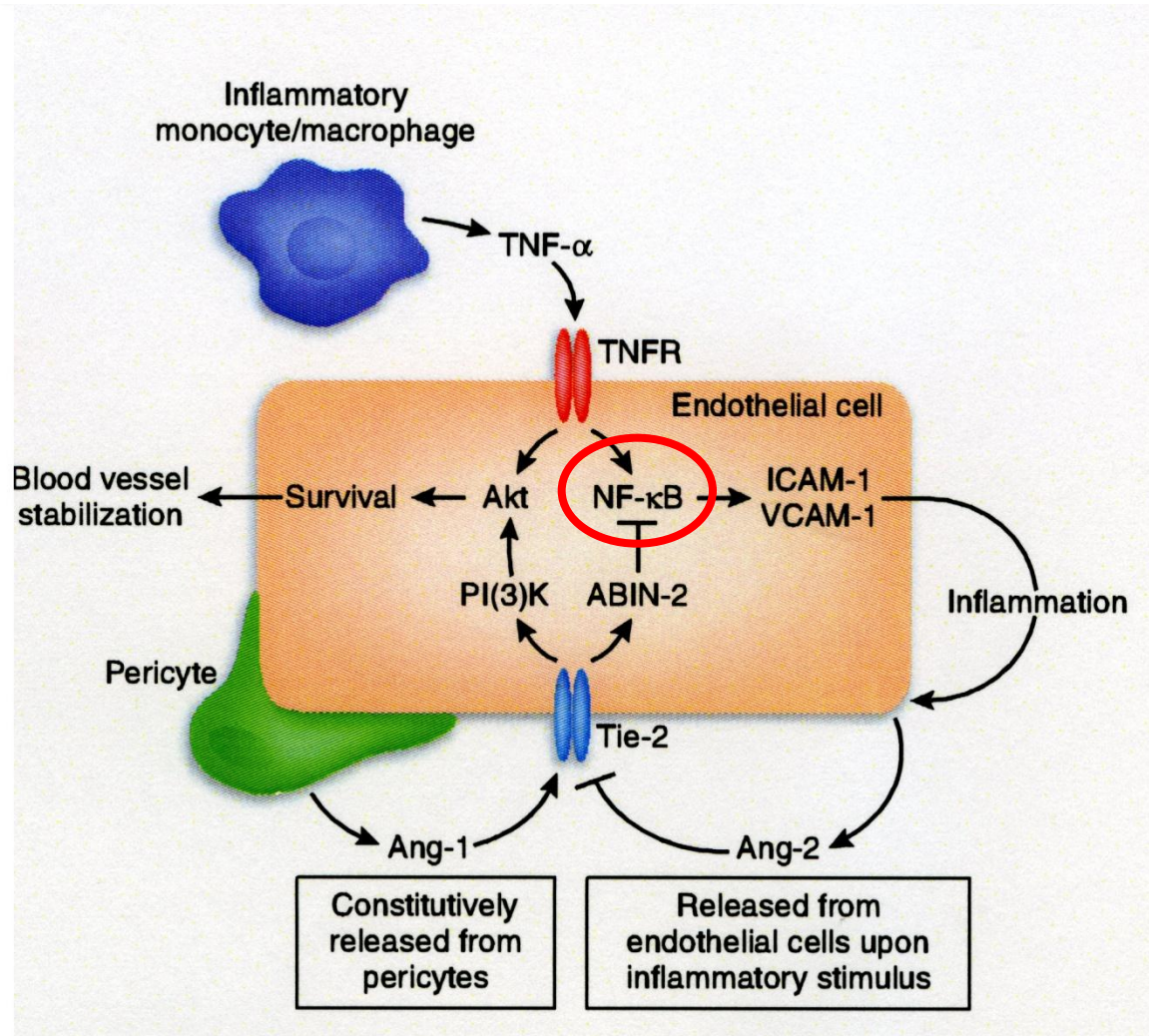


# Polyphenols effects on TNF $\alpha$ expression

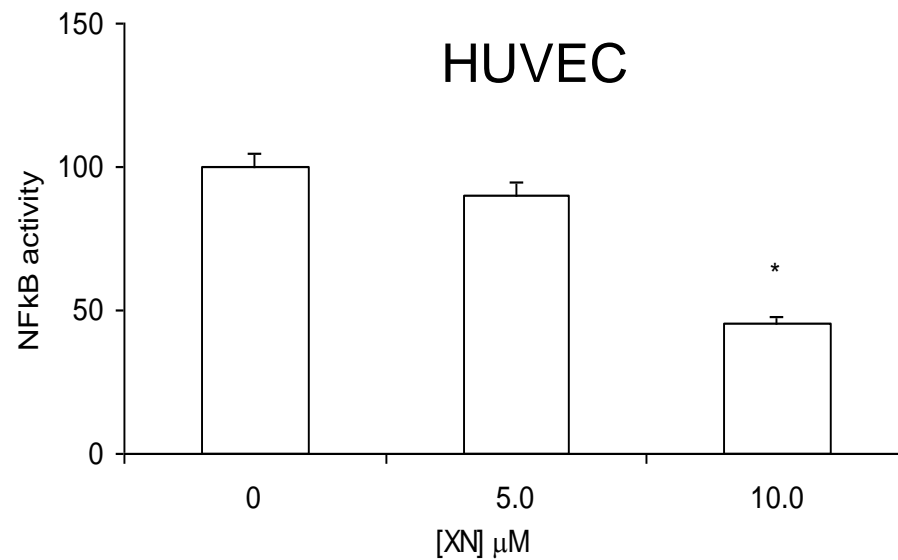


ELISA assay

# NFκB promotes inflammation and angiogenesis in endothelial cells

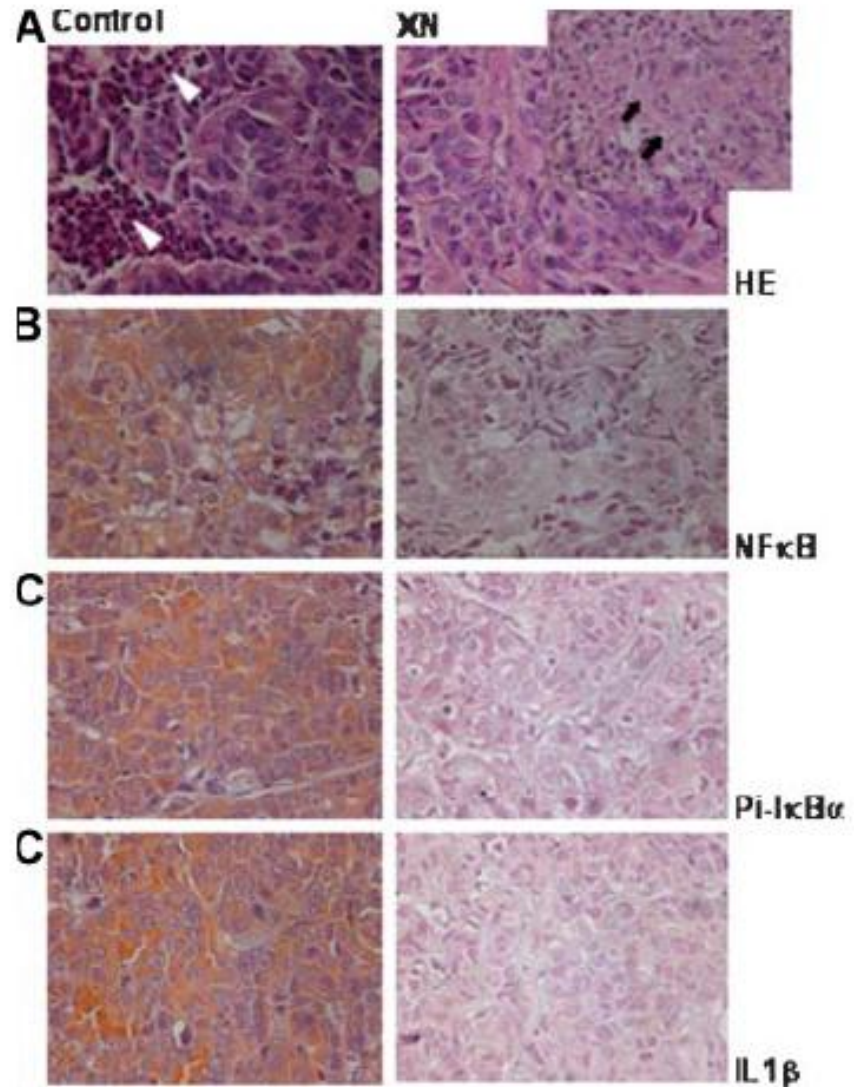
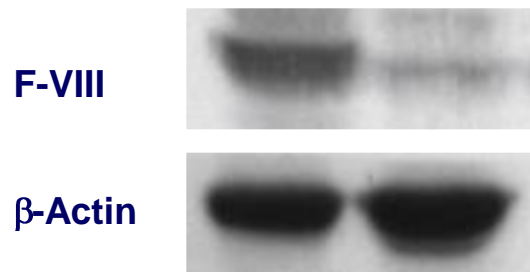
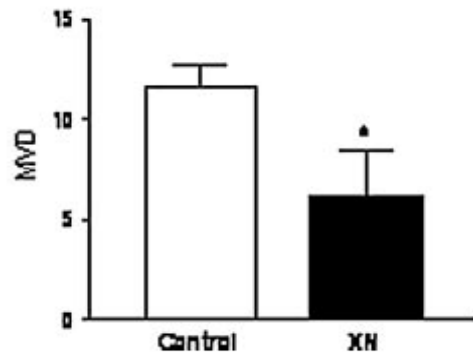
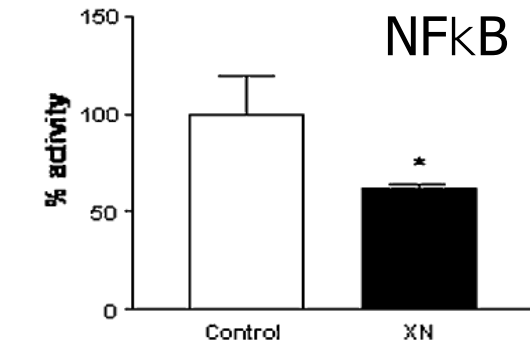


# XN prevents angiogenesis *in vitro* (HUVEC) and inhibits NFκB activity

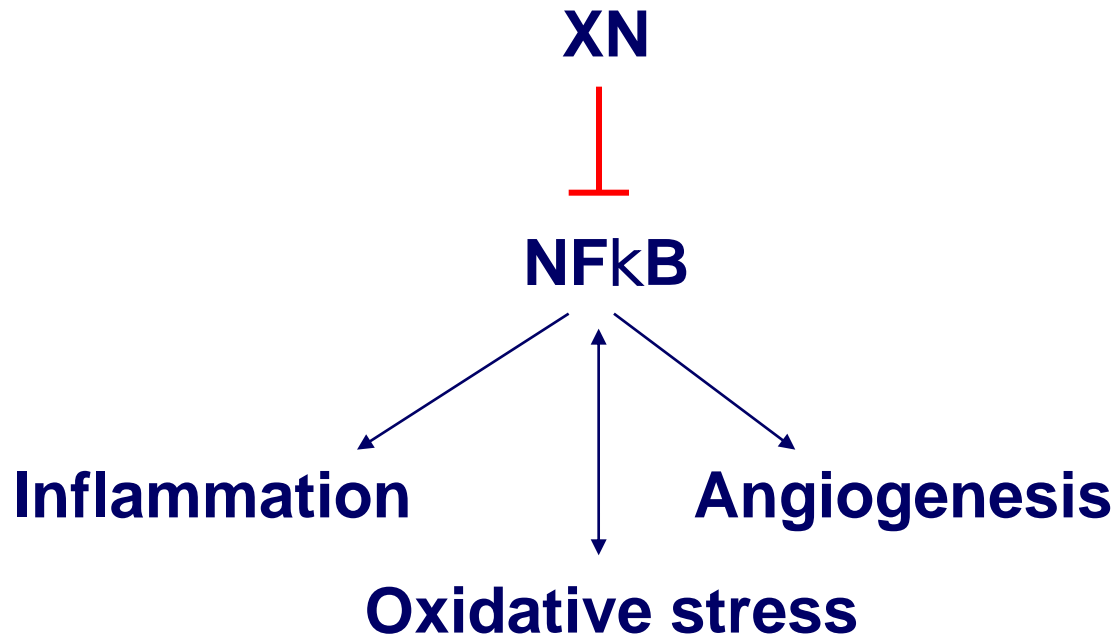




# XN inhibits inflammation and angiogenesis in *in vivo* breast carcinoma



# XN impairs inflammation and angiogenesis in *in vivo* breast cancer through NFkB



# Do platelets contribute to the angiogenic phenotype?

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## OPINION

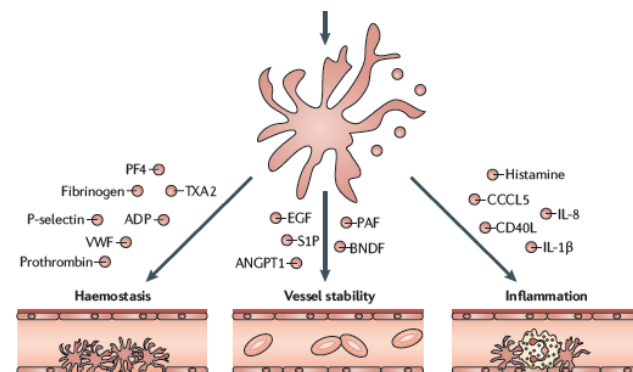
# Contribution of platelets to tumour metastasis

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*Laurie J. Gay and Brunhilde Felding-Habermann*

Abstract | Extensive experimental evidence shows that platelets support tumour metastasis. The activation of platelets and the coagulation system have a crucial role in the progression of cancer. Within the circulatory system, platelets guard tumour cells from immune elimination and promote their arrest at the endothelium, supporting the establishment of secondary lesions. These contributions of platelets to tumour cell survival and spread suggest platelets as a new avenue for therapy.

## CORRESPONDENCE



# Could platelet-accumulating polyphenols prevent tumour metastasis?

Rita Negrão, Delfim Duarte, Raquel Costa and Raquel Soares

We read with great interest the Review by Gay and Felding-Habermann (Contribution of platelets to tumour metastasis. *Nature Rev. Cancer* 11, 123–124 (2011))<sup>1</sup>, which discussed the observation that cancer patients usually present signs of throm-

We have been studying the effects on angiogenesis and inflammation of a group of naturally derived compounds, polyphenols, which have established anti-oxidant, anti-inflammatory, anti-angiogenic and antitumour properties. The topical administration of

# Conclusions

Beer-derived polyphenols (XN and IXN) impair oxidative stress, inflammation and angiogenesis, three processes associated with cancer progression.

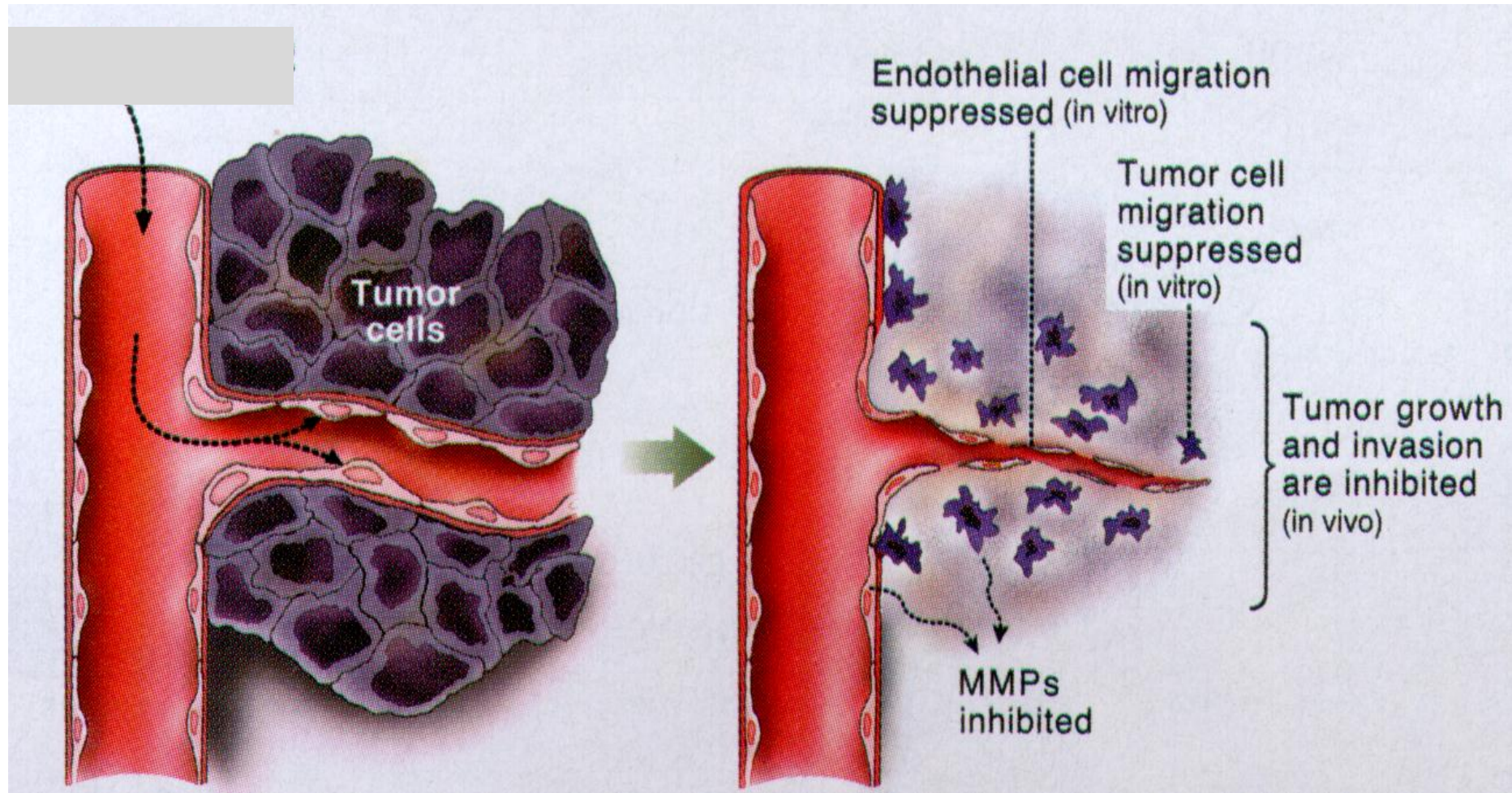
Therefore, they might be helpful cancer therapeutic/preventive agents.

# Take-home message

- Angiogenesis is a crucial event in cancer
- Estrogens lead to normalized vessels
- Some cancer therapy agents also affect vascular wall cells
- Angiogenesis strongly associates with inflammation and oxidative stress in cancer (and other diseases)
- Anti-inflammatory and anti-oxidant agents (e.g. polyphenols) might be useful in controlling tumor angiogenesis

# Tumor angiogenesis: therapeutic implications

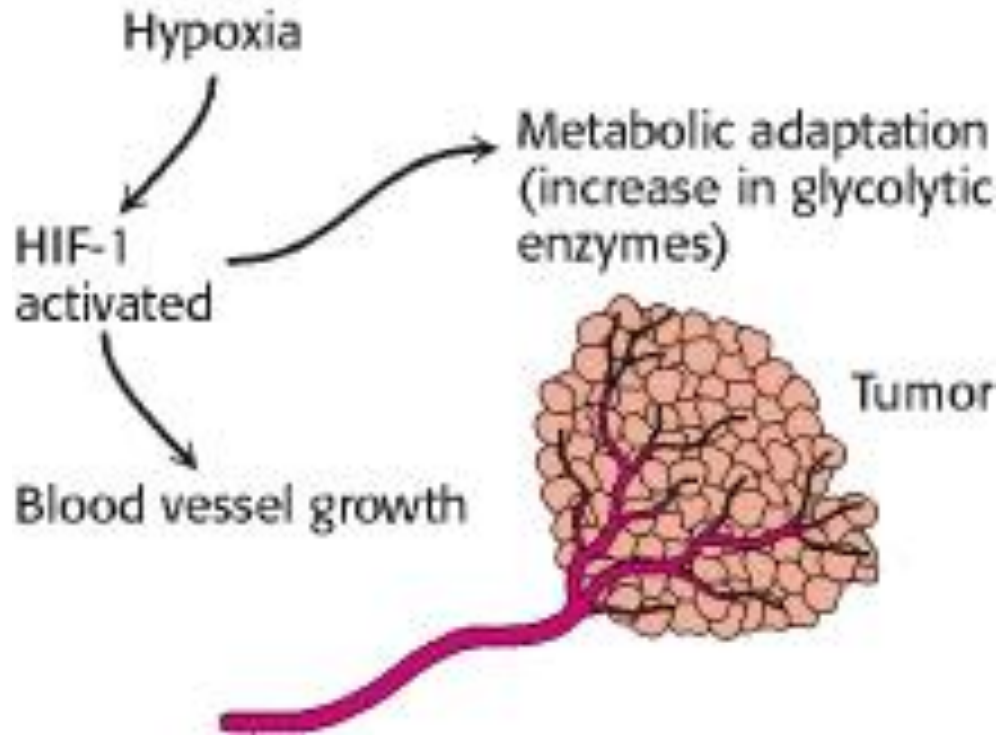
*J Folkman. N Engl J Med, 1971; 285: 1182-6.*



Angiogenesis blockage can be a good strategy to prevent tumor growth

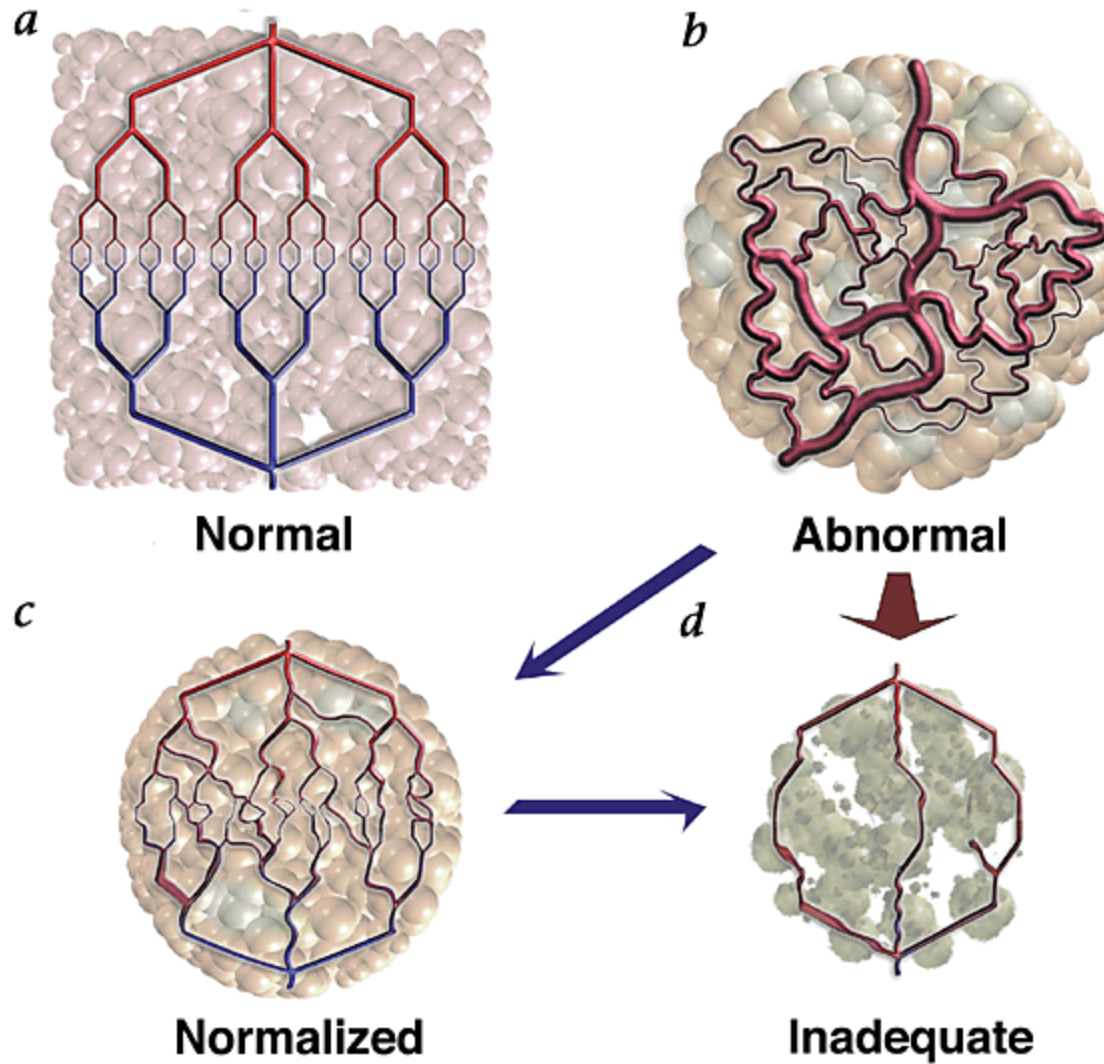
# Angiogenesis inhibition results in hypoxia

## Hypoxia induces Angiogenesis





# Normalizing vs inhibiting vascularization



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